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(54) Title: VIRAL PHOSPHOLIPASE A_2 ENZYMES, ANTI-VIRAL AGENTS AND METHODS OF USE

(57) Abstract: The present invention provides a novel class of phospholipase A_2 enzyme (PLA_2) from viruses and a method for identifying, isolating, purifying and characterizing enzymes of this class. The present invention includes viral PLA_2 proteins, nucleic acids, and antisense oligonucleotides, and the use of these molecules in screening methods for anti-viral agents, in decreasing the infectivity and/or replication of viruses and as research tools. The present invention further includes treatment or prevention of virus-associated diseases using viral PLA_2 inhibitors and the use of the viral PLA_2-encoding region to improve virus-based gene therapy vectors.

VIRAL PHOSPHOLIPASE A_2 ENZYMES, ANTI-VIRAL AGENTS AND METHODS OF USE

FIELD OF THE INVENTION

The present invention pertains to a class of phospholipase A_2 proteins and nucleic acids and their use. More particularly the present invention pertains to viral phospholipase A_2 proteins and nucleic acids and their use.

BACKGROUND OF THE INVENTION

Phospholipase A_2

Phospholipase enzymes catalyze the removal of fatty acid residues from phosphoglycerides. Phospholipases A_2 (PLA_2s) are a superfamily of key enzymes involved in a multitude of (patho)physiological and cellular processes, including lipid membrane metabolism, signal transduction pathways, inflammation, acute hypersensitivity, and degenerative diseases (Balsinde *et al.,* (1999) *Annu. Rev. Pharmacol. Toxicol.* **39**:175-189; Kramer and Sharp (1997) *FEBS Letters* **410**:49-53; Nishizuka (1992) *Science* **258**:607-614; Dennis (1994) *J. Biol. Chem.* **269**:13057-13060; Dennis (1997) *Trends Biochem. Sci.* **22**:1-2; Yuan and Tsai (1999) *Biochim Biophys Acta* **1441**:215).

PLA_2s hydrolyze the ester bonds at the *sn*-2 position of the glycerol moiety of membrane phospholipids to yield lysophospholipids and fatty acids, from which secondary messengers may be generated. These secondary messengers may modulate enzyme activities, ion channels, and (post-)transcription factors (Nishizuka (1992) *Science* **258**:607-614; Rao *et al.,* (1993) *Oncogènes* **8**:2759-2764; Yao *et al.,* (1995) *Nature* **378**:307-310).

PLA_2s are found in many living species and form a diverse family of enzymes. The main groups of PLA_2s are the cytosolic PLA_2s, the Ca^{2+}-independent PLA_2s, and the secretory PLA_2s ($sPLA_2$s). A large number of $sPLA_2$s, from host cells as well as from snake and bee venoms, have been structurally characterized, and show a high degree of sequence homology (See, for example, Chang *et al.,* (1987) *Biochemical Pharmacology* **36**:2429-2436). Although

the known $sPLA_2$s were originally divided into groups by source organism and their primary amino acid sequences, they are now characterized by a growing list of other attributes, such as the number and position of disulfide bonds. Group I includes pancreatic $sPLA_2$s from vertebrates, including mammals, and snake venom $sPLA_2$s. These $sPLA_2$s are active at pH 6-8 and their activity is dependent on the presence of calcium ion (Ca^{2+}). Group II contains a mixture of non-pancreatic, or synovial, $sPLA_2$s and $sPLA_2$s from the venom of crotalids and viperids. Both group I and group II members have a similar 3D-structure. Group III contains $sPLA_2$s from bee venoms. $sPLA_2$s from all three groups have a 14 kDa molecular mass and are disulfide-rich (Dennis (1994) *J. Biol. Chem.* **269**:13057-13060; Dennis (1997) *Trends Biochem. Sci.* **22**:1-2). While all low-molecular weight (~13.5-16.8 kDa), Ca^{2+}-dependent PLA_2s possess secretion sequences and have been found extracellularly, some are also cell-associated (Anderson *et al.* (1994) *Prostaglandins, Leukotrienes and Essent Fatty Acids* **51**:19; Fayard *et al.* (1998) *J Cell Sci* **111**: 985). Other types of PLA_2s include group V PLA_2 (Chen *et al.* (1994) *J. Biol. Chem.* **269**:2365), and group X PLA_2 isoforms (Valentin *et al.* (1999) *J. Biol. Chem.* **274**: 31195).

The product of $sPLA_2$ activity, arachadonic acid, is processed into bioactive lipid mediators or shuttled into pathways for the synthesis of eicosanoids. In mammals eicosanoids, such as prostaglandins, prostacyclins, thromboxanes and leukotrienes, are involved in pain, inflammation and fever. As such, inhibitors of mammalian $sPLA_2$ have been described for use in the treatment of a variety of inflammatory disorders (for example, see International Patent Application No. WO 96/27604, European Patent Application No. EP 950 657 A2 and U.S. Patent No. 5,948,626). In addition, a recent report has shown that $sPLA_2$ isolated from bee or snake venom inhibits human immunodeficiency virus (HIV) by blocking viral entry into host cells (Fenard *et al.*, (1999) *J. Clin. Invest.* **104**:611).

While prokaryotic versions of PLA_2 have been reported in bacteria such as *Escherichia coli* and *Streptomyces violaceoruber*, viral PLA_2 has not previously been identified.

Viruses

Viruses are infectious agents that are found in virtually all life forms, including humans,

animals, plants, fungi, and bacteria. Viruses often damage or kill the cells that they infect, causing disease in infected organisms. The difficulty in developing anti-viral therapies stems from the large number of variant viruses that can cause the same disease, as well as the inability of drugs to disable a virus without disabling healthy cells. The development of specific anti-viral agents, therefore, is a major focus of current research.

Therapeutic Anti-Viral Treatments

Although a number of methods exist currently for the treatment of viral infections, many of these are complicated by the side effects the anti-viral agent(s) has on the host system.

One example of a method for inhibiting a viral infection is provided by U.S. Patent No. 5,449,608, which describes a method of inhibiting the human parvovirus, B19. The method comprises administering compositions containing a B19 binding domain, thus preventing binding of B19 to host cells. Unfortunately, such an agent competes with similar molecules already present in the host cells, without a clear competitive edge, and is prone to catalytic breakdown.

Similarly, a soluble form of the receptor (CD-4) for the human immunodeficiency virus, HIV-1, has been shown to be somewhat effective in reducing the infectivity of HIV in tissue culture, but this result could not be replicated *in vivo*. This is likely due to the large number of receptor binding sites present on a single virus particle, thus making it difficult for a soluble receptor to block all sites and prevent binding of the virus to cells (see Flint *et al.*, *Principles of Virology* (Washington, DC: ASM Press, 1999) 119).

Clearly, a need remains to provide easily applicable methods and agents that may be used to effectively treat viral infections, particularly in cases where vaccination has failed or where subjects were not vaccinated.

The present invention provides a means of inhibiting virus infection and/or replication in animals, including humans, and of treating persistent infections and associated syndromes.

This background information is provided for the purpose of making known information believed by the applicant to be of possible relevance to the present invention. No admission is necessarily intended, nor should be construed, that any of the preceding information constitutes prior art against the present invention.

SUMMARY OF THE INVENTION

An object of the present invention is to provide viral phospholipase A_2 enzymes, antiviral agents and methods of use. In accordance with an aspect of the present invention, there is provided a viral polypeptide that has phospholipase A_2 activity.

In accordance with another aspect of the present invention, there is provided an isolated polynucleotide encoding a viral polypeptide that has phospholipase A_2 activity, antisense oligonucleotides complementary to the polynucleotides, vectors comprising the polynucleotides, and host cells genetically engineered with the polynucleotides or vectors.

In accordance with another aspect of the present invention there is provided methods of decreasing parvovirus infection and replication, and thereby methods of treating parvovirus-related diseases.

In accordance with another aspect of the present invention viral PLA_2s are used in manufacturing, clinical, and research settings. At present, bee venom is commonly used as a standard in measuring PLA_2 activity. The viral PLA_2 of porcine parvovirus (PPV), however, has a 40 to 100-fold higher specific activity than bee venom. This high activity makes PPV PLA_2 ideal for use as a PLA_2 standard.

In accordance with another aspect of the present invention there is provided methods for screening compounds in order to identify inhibitors of viral PLA_2 activity, comprising adding a candidate inhibitor compound to a solution containing a viral PLA_2 and a phospholipid substrate, and detecting the inhibition of PLA_2 activity.

In accordance with another aspect of the present invention there is provided antisense oligonucleotides designed to inhibit expression of viral PLA_2.

In accordance with a further aspect of the present invention is the use of these viral PLA_2 inhibiting agents as anti-viral agents in the treatment of various viral infections.

In accordance with still another aspect of the present invention there is provided the use of viral PLA_2 nucleic acids, or variations thereof, to improve gene-therapy vectors.

Various other objects and advantages of the present invention will become apparent from the detailed description of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts a parvovirus genome.

The right-half of the parvovirus genome contains a large ORF coding for a nested set of capsid proteins (VPs) from alternative in-frame initiation codons so that these VPs differ by their *N*-terminal extensions. The VPs can be subdivided into 3 domains: common *C*-terminal domains required for capsid formation (60 copies per capsid); a small hinge-(like) domain; and unique *N*-terminal extensions. The capsid-forming domain consists of a β-barrel in which β-strands (black boxes: αA and αB are helices) are connected by loops (L1-4). About 100 amino acids upstream of the start of VP is a conserved domain (cd). The shaded boxes represent the domains (V1ups) cloned in expression vectors (VP1up: PPV-VP1up amino acids 2-174 [SEQ ID NO: 50]; B19-VP1up amino acids 2-240 [SEQ ID NO: 52]; and *Gm*DNV-VP1up amino acids 1-378 [SEQ ID NO: 54]).

Figure 2 presents a protein sequence alignment of parvovirus VP1up regions and representatives of $sPLA_2$ groups. Parvoviral and $sPLA_2$ sequences are separated by numbers of the common structural numbering for group I/II $sPLA_2$ (Renetseder *et al.*, (1985) *Annu. Rev. Pharmacol. Toxicol.* **39**:175; Kramer and Sharp (1997) *FEBS Lett.* **410**:49; Nishizuka (1992) *Science* **258**:607). Black shading indicates 100% identity among sPLA2s. Dark grey

indicates 100% identity among vPLA2s. Medium grey shading indicates 100% identity among all PLA_2s. GenBank accession numbers are indicated except for those not yet deposited.

Figure 3 demonstrates the specificity of PLA_2 activity.

A. Thin layer chromatography after hydrolysis of phosphatidylcholine substrate (PL) into fatty acid (FA) and lysophosphatidylcholine (LPL) by expressed $pvPLA_2$ in the mixed micelles assay. PLA_2s used were: lanes 1, 2 and 5 (0.6 ng of PPV PLA_2), lanes 3 and 4 (250 ng of B19 PLA_2), lane 6 (350 ng of *Gm*DNV PLA_2), lane 7 (2,000 ng thioredoxin as negative control) and lane 8 (15 ng bee venom PLA_2). The 3C9 monoclonal antibody, which binds to the *C*-terminus of PPV-VP1up, reduced PPV PLA_2 activity of VP1up (lane 2), but not that of B19 (lane 4). Adding EGTA to a final concentration of 5 mM abolished PLA_2 activity (lane 5).

B. Sequences outside the conserved $pvPLA_2$ domain increased $pvPLA_2$ activity of the PLA_2 motif of expressed PPV-VP1ups. VP1up from M1 to S174 was used throughout this work and was assigned the relative specific activity of 1.0. Sequences within this expressed peptide but outside the PLA_2 motif (dark-grey) contributed to the activity as shown by the relative specific activities.

C. The impact of Ca^{2+} concentration, pH and different substrates on the activity of $pvPLA_2$. Since 1 mM Ca^{2+}, 50 mM Tris-HCl, pH 8.0, and phosphatidylcholine (PC) were used in standard assays, these were set at 100% relative activity. PE = phosphatidylethanolamine, PI = phosphatidylinositol. Samples were measured in triplicate.

D. PLA_2 activity of untreated 0.2 µg virions (lane 1) and after dissociation (lanes 2 & 3) and heat shock (lanes 4 & 5). Bee venom was included as a positive control (lanes 6 & 7). Lane 8 contains the negative control. Samples in lanes 3, 5 and 7 were treated with anti-VP1up antibody.

Figure 4 depicts the predicted 3D-structure of the $vPLA_2$ domain. The predicted 3D-structure of the $vPLA_2$ domain, as shown for PPV/B19, showed sequence homology with group III $sPLA_2$ at the – and *C*-termini, whereas the centre section resembled group I/II $sPLA_2$ (sequence of IB pancreatic $sPLA_2$ shown). The top line shows the position of helices (H) for group I/I, which are inferred from the homologous porcine pancreatic PLA_2. The bottom line shows the position of helices (H) for group III PLA_2. The predicted three-dimensional model of B19/PPV PLA_2 was obtained by homology modeling using the program MOE. Both the three-dimensional structure of bee venom PLA_2 (Group III, in magenta) and the three-dimensional structure of the pancreatic porcine PLA_2 (Group I/II, in orange) were used for homology modeling of the $vPLA_2$ (centre). Note that residues L, V, I as well as residues Y, F, W were considered equivalent residues. The three-dimensional images were generated with the program GRASP. The color code in the sequence matches that in the $vPLA_2$ structure. Residues coloured green in $vPLA_2$ indicate residues that are conserved in both group I/II and group III PLA_2s; orange-coloured residues are conserved in group I/II; and magenta-coloured residues are conserved in group III PLA_2s. The amino acids identified in the $vPLA_2$ structure were submitted to site-directed mutagenesis.

Figure 5 depicts immunofluorescence of infected cells using wild-type (wt) or mutant virus, with or without NH_4Cl treatment. Immunofluorescence was used to follow the entry and infection of PT cells by the wt or mutant virus. At 0 hours of entry, a strong staining, often patchy, was observed on the cellular membrane. After 4 hours, virus entry led to a cytoplasmic, granular, and perinuclear staining, whereas with NH_4Cl treatment, the virus was still mostly distributed throughout the cytoplasm. After 8 hours, all incoming virus was perinuclear except for wt without NH_4Cl, for which some nuclei became positive. At 12 hours, only wt without NH_4Cl yielded significant numbers of positive nuclei. Some positive nuclei appeared at 18 hours for wt with NH_4Cl, and for the P21W or P21L mutants. The delivery of the viral genome can be seen to be impaired in the case of the mutant virus, and is further inhibited by treatment with NH_4Cl.

Figure 6 shows the binding (**A**) and entry (**B**) of ^{35}S-labelled porcine Parvovirus using $4x10^5$ cells and $5x10^9$ wild type (wt) virus particles. Equivalent amounts of mutant viruses were

used for the binding studies, and twice as much for the entry studies (experiments in quadruplicate).

C. ^{35}S-labeled PPV particles (wt or mutant) were added to $2x10^7$ cells in Petri dishes and about 20% was recovered in the cells. After cellular fractionation, the distribution of virus over cytosolic and nuclear fractions was measured by liquid scintillation. The relative radioactivity is calculated as follows: [nuclear fraction-bound activity]/[cellular fraction-bound activity]. Standard errors were calculated from three experiments.

D. Co-localization of PPV and LAMP-2 was studied by confocal microscopy. Anti-PPV antibodies were labeled with FITC, giving green fluorescence, and anti-LAMP-2 antibodies with Texas Red, giving red fluorescence. Co-localization of PPV and LAMP-2 resulted in yellow staining.

Figure 7 demonstrates distribution of viral DNA in infected cells. Incoming viral DNA was detected in the cytoplasm by *in situ* hybridization 4 (**A**) and 8 (**B**) hours post-infection (p.i.), whereas replicating DNA in the nucleus was found at 12 hours p.i. (**C**). At 12 hours p.i., no DNA could be detected in the nucleus in the case of the HD (**D**) and $P21^{PVW}$ mutants. Aphidicoline, a DNA polymerase inhibitor, also prevented the appearance of viral DNA in the nucleus (**F**). The $sPLA_2$ inhibitors tetracain (TC), and oleyloxyethyl phosphorylcholine (OP), also reduced the number of nuclei containing viral DNA (**G**).

Figure 8 shows nucleic acid sequences of cloned V1up regions of porcine parvovirus [SEQ ID NO: 50], B19 human parvovirus [SEQ ID NO: 52], and *Galleria mellonella* densovirus [SEQ ID NO: 54].

Figure 9 shows amino acid sequences of cloned V1up regions of porcine parvovirus [SEQ ID NO: 49], B19 human parvovirus [SEQ ID NO: 50], and *Galleria mellonella* densovirus [SEQ ID NO: 52].

Figure 10 shows the 5234 base pair nucleic acid sequence of *Acheta domesticus* densovirus [SEQ ID NO: 65].

Figure 11 shows the nucleic acid sequence of the genomic region containing the PLA_2 motif for *Acheta domesticus* densovirus [SEQ ID NO: 64] and *Mythimna loreyi* densovirus [SEQ ID NO: 62].

Figure 12 shows the amino acid sequence of the region containing the PLA_2 motif for *Acheta domesticus* densovirus [SEQ ID NO: 63] and *Mythimna loreyi* densovirus [SEQ ID NO: 61]. The conserved histidine (H) and aspartic acid (D) residues are highlighted.

Figure 13 shows the sequences identified by phage display that bind to viral PLA_2.

DETAILED DESCRIPTION OF THE INVENTION

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. For the purposes of the present invention, the following terms and abbreviations are defined below.

The term "agent" is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials such as bacteria, plants, fungi, or animal (particularly mammalian) cells or tissues.

The term "amino acid motif" is used herein to denote a characteristic sequence of amino acids the presence of which can serve to identify members of a family of proteins. It is understood by those of skill in the art that variations of one or two amino acids within a motif does not preclude a protein from belonging to the family defined by the motif.

This invention is based on the unexpected finding that PLA_2 activity is present in the capsids of viruses. This invention is also based on the unexpected determinations that this viral PLA_2 activity is essential for infection and that inhibition of this PLA_2 activity decreases the infectivity and/or replication of the viruses.

Characterization of Viral PLA_2s

The present invention describes a class of PLA_2s found in viruses. These viral PLA_2s have significant differences from known PLA_2s, including structure (Figure 4), biological function, and amino acid sequences (Figure 2); thus, these viral PLA_2s form a new group of PLA_2s within the PLA_2 superfamily.

The present invention provides viral PLA_2s containing a unique, characteristic sequence profile. Viral PLA_2s comprise the amino acid motif [WY]-[CIVL]-G-x-[GP]-[GKNS]-x(8,14)-D-x(2)-[AC]-x(2)-H-D-x(2)-Y, where the notation [WY] represents alternative amino acids at this position, x is any amino acid, x(2) represents a stretch of 2 amino acids and x(8,14) represents a stretch of between 8 and 14 amino acids. The above motif is characteristic of viral PLA_2 sequences.

In accordance with one aspect of the present invention, there is provided polynucleotides that encode viral PLA_2s containing the amino acid motif [WY]-[CIVL]-G-x-[GP]-[GKNS]-x(8,14)-D-x(2)-[AC]-x(2)-H-D-x(2)-Y. The present invention further relates to polypeptides which contain the viral PLA_2 amino acid motif and exhibit phospholipase A_2 activity.

In one embodiment of the present invention, the polypeptide is a variant of a naturally occurring viral PLA_2 which may have a substitution, deletion, or addition of one or more amino acids. In a related embodiment, there is provided polynucleotides that encode such variants.

The present invention further provides variants of the viral PLA_2s that may be inactive due to the substitution of an active site residue, or that may contain slight variations of the above amino acid motif. Analogous variants exist in the mammalian PLA_2s in the form of pseudogenes. The viral PLA_2s of the present invention may be obtained from any virus presently identified and from viruses yet to be identified or may be prepared by recombinant molecular biology techniques or chemical synthesis.

1) PLA_2 activity

The viral PLA_2s of the present invention are ultimately defined by their PLA_2 activity. PLA_2 activity is defined as the ability to hydrolyse the fatty acid from the *sn*-2 position of phospholipids to yield lysophosphatidyl compounds.

One exemplary assay to determine PLA_2 enzyme activity is described. PLA_2 enzyme activity can be established using the *E. coli* radioactive assay (Elsbach and Weiss (1991) *Methods Enzymol* 197:24). *E. coli* phospholipids are metabolically labeled for 3 hours with [^{14}C]-oleic acid (95% in *sn*-2 position). The bacteria are then autoclaved for 15min. to inactivate bacterial phospholipases, and washed. The viral sample (1-1000 pg) is added to the bacteria (10,000 cpm) in standard buffer (50 mM Tris-HCl, pH 8.0, and 10 mM $CaCl_2$) and incubated for 30min. at 37°C. The reaction is stopped by the addition of ice-cold 1% BSA. After centrifugation, 50% of the supernatant is counted. PLA_2 activity is identified and/or quantified by the release of radioactive fatty acids from the phospholipids into the supernatant.

A mixed micelles assay can also be used to establish PLA_2 activity. The substrates used are 6 μM of either L-3-phosphatidylcholine,1,2-di[1-^{14}C]oleoyl (specific activity 110 mCi/mmol), L-3-phosphatidylinositol L-1-stearoyl-2-[1-^{14}C]arachidonyl (specific activity 48 mCi/mmol), or phosphatidylethanolamine L-1-palmitoyl,2-[1-^{14}C]arachidonyl (specific activity 54.6 mCi/mmol). The assay is based on that described by Manjunath *et al.*, (1994) *Biochem J.* 303:121, with the following modifications: 1 mM Triton X-100 is used instead of deoxycholate; the total reaction volume is 50 μl and the reaction is stopped after 10 min by adding 80 μl chloroform/methanol (2:1) and 50 μl of saturated KCl solution. Separation is on silica gel thin layer chromatography (TLC) plates. Optimal separation of the phosphatidylcholine reaction products is obtained using a solvent solution containing chloroform, methanol and water at a ratio of 65:35:4. For phosphatidyl ethanolamine and phosphatidylinositol, chloroform and methanol (87:13) are used as a solvent. The separated products are quantified using a Molecular Dynamics PhosphorImager SI after drying. PLA_2 activity is identified and/or quantified by the presence of radioactive fatty acids, released from phospholipids, on each TLC plate.

2) Parvoviral PLA_2s

In one embodiment of the present invention, the viral PLA_2 is a parvovirus PLA_2. In a related embodiment the parvovirus PLA_2 is present in the VP1-unique part (VP1up) of the largest capsid protein of parvoviruses.

In accordance with the present invention, the parvovirus PLA_2s comprise the following, more specific version of the above amino acid motif: Y-x-G-P-G-x(12)-D-x(2)-A-x(2)-H-D-x(2)-Y, where x is any amino acid, x(2) represents a stretch of 2 amino acids, x(12) represents a stretch of 12 amino acids and wherein one of the specified amino acids Y, G, P, G, D, A, D, or Y may be exchanged singly for any other amino acid. This invention includes viral PLA_2s obtained from known parvoviruses, including, but not limited to, mammalian parvoviruses, such as canine parvovirus (CPV), mink enteritis virus (MEV), porcine parvovirus (PPV), bovine parvovirus (BPV), goose parvovirus (GPV), feline panleukopenia virus (FPLV), the human parvoviruses, such as B19, V9, and RA-1, and insect parvoviruses, such as *Gm* DNV; *Ml* DNV; *Jc* DNV; *Pi* DNV; *Ds* DNV; *Cp* DNV; *Ce* DNV; *Ad* DNV; *Pf* DNV; and *Bm* DNV. The parvoviral PLA_2s of the present invention may also be obtained from parvoviruses yet to be identified. The protein sequences determined for 30 different parvoviral PLA_2s are shown in Figure 2.

Preparation of Viral PLA_2s

Unless otherwise specified, the viral PLA_2 proteins, peptides or fragments thereof, of the present invention are prepared in such a manner that their intrinsic enzymatic activity is retained. Further, amino acid residues may be deleted, added or substituted for those that appear in the amino acid sequences of the viruses of interest in instant invention. It should also be appreciated that the present invention contemplates amino acid sequences that are equivalent to, or constitute active fragments of, the amino acid sequences for the viral PLA_2 enzymes of the present invention.

The proteins of the present invention can be prepared through the use of recombinant techniques, or from viral extracts. In general, viral PLA_2 proteins according to the instant invention can be produced by transformation (transfection, transduction, or infection) of a host cell with all or part of a viral PLA_2-encoding DNA fragment in a suitable expression vehicle. Suitable expression vehicles include: plasmids, phagemids, viral particles, and phage. The entire expression vehicle, or a part thereof, can be integrated into the host cell genome. In some circumstances, it is desirable to employ an inducible expression vector.

Those skilled in the field of molecular biology will understand that any of a wide variety of expression systems can be used to provide a recombinant viral PLA_2 protein of the instant invention. The precise host cell used is not critical to the instant invention. A viral PLA_2 protein can be produced in a prokaryotic host (e.g., *E. coli* or *B. subtilis*) or in a eukaryotic host (e.g., *Saccharomyces* or *Pichia*; mammalian cells, e.g., COS, NIH 3T3, CHO, BHK, 293, or HeLa cells; or insect cells) using techniques that are standard in the field of molecular biology.

The methods of transformation or transfection and the choice of expression vehicle will depend on the host system selected. Transformation and transfection methods are described, e.g., in Ausubel *et al.* (1994) Current Protocols in Molecular Biology, John Wiley & Sons, New York. Expression vehicles can be chosen from those provided, *e.g.* in Cloning Vectors: A Laboratory Manual (Pouwels *et al.*, 1985, Supp. 1987).

The host cells harbouring the expression vehicle can be cultured in conventional nutrient media adapted as necessary for activation of a chosen gene, repression of a chosen gene, selection of transformants, or amplification of a chosen gene. One example of an expression system is the mouse 3T3 fibroblast host cell transfected with a pMAMneo expression vector (Clontech, Palo Alto, Calif.). pMAMneo provides an RSV-LTR enhancer linked to a dexamethasone-inducible MMTV-LTR promotor, together with an SV40 origin of replication, which allows replication in mammalian systems, a selectable neomycin gene, and SV40 splicing and polyadenylation sites. DNA encoding a viral PLA_2 protein can be inserted into the pMAMneo vector the appropriate orientation to allow expression of the protein. The

recombinant viral PLA_2 protein can then be isolated from the expression system using standard techniques. Other host cells that can be used in conjunction with the pMAMneo expression vehicle include COS cells and CHO cells (ATCC Accession Nos. CRL 1650 and CCL 61, respectively).

Alternatively, viral PLA_2 polypeptides can be produced as fusion proteins. For example, the expression vector pUR278 (Ruther *et al.* (1983) *EMBO J.* 2, 1791) can be used to create lacZ fusion proteins, or the pGEX vectors can be used to create glutathione S-transferase (GST) fusion proteins. In general, GST-fusion proteins are soluble and can be easily purified from lysed cells by adsorption to glutathione-agarose beads, followed by elution in the presence of free glutathione. The pGEX vectors are designed to provide thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

Specific initiation signals may also be required for efficient translation of inserted nucleic acid sequences. These signals include the ATG initiation codon and adjacent sequences. In cases where an entire native viral PLA_2 gene or cDNA, including its own initiation codon and adjacent sequences, is inserted into the appropriate expression vector, no additional translational control signals may be needed. In other cases, exogenous translational control signals, including, perhaps, the ATG initiation codon, must be provided. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. If necessary, the efficiency of expression can be enhanced by the inclusion of appropriate transcription enhancer elements and / or transcription terminators (Bittner *et al.* (1987) *Methods in Enzymol.* 153, 516).

Alternatively, a viral PLA_2 protein can be produced from a stably-transfected mammalian cell line. A number of vectors suitable for stable transfection of mammalian cells are available to the public, see, *e.g.* Pouwels *et al.* (*supra*). Methods for constructing such cell lines are also publicly available and known to those skilled in the art, see for example Ausubel *et al.*

(*supra*). In one example, cDNA encoding the viral PLA_2 protein can be cloned into an expression vector that includes the dihydrofolate reductase (DHFR) gene. Integration of the plasmid and, therefore, the viral PLA_2 protein-encoding gene into the host cell chromosome can then be selected for by including an appropriate amount of methotrexate in the cell culture medium (as described in Ausubel *et al., supra*). This dominant selection can be accomplished in most cell types.

Fusion proteins can be readily purified by utilising an antibody or other binding partner specific for the fusion protein being expressed. For example, a system described in Janknecht *et al.* (1981) *Proc. Natl. Acad. Sci. USA* **88**:8972, allows for the ready purification of non-denatured fusion proteins expressed in human cell lines. In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. Extracts from cells infected with the recombinant vaccinia virus are loaded onto a Ni^{2+} nitriloacetic acid-agarose column, and the histidine-tagged proteins are selectively eluted with imidazole-containing buffer.

Alternatively, a viral PLA_2 or a portion thereof, can be fused to an immunoglobulin Fc domain. Such a fusion protein can be readily purified using a protein A column.

It will be apparent to one skilled in the art that the viral PLA_2 peptides disclosed herein can be created by designing nucleic acid sequences that encode for viral PLA_2, but which differ, by reason of the redundancy of the genetic code, from the sequences disclosed herein. Accordingly, the degeneracy of the genetic code further enables major variations in the nucleotide sequence of a nucleic acid molecule but does not broaden the scope of the present invention since the amino acid sequence of the encoded protein remains unchanged. Based upon the degeneracy of the genetic code, variant nucleic acid sequences may be derived from the nucleic acid sequences disclosed herein. These variant nucleic acid sequences can be produced by modifying or synthesizing nucleic acid sequences. Variant nucleic acid sequences include deletion, addition, substitution, or a combination thereof, of different nucleotides.

A viral PLA_2 protein sequence of the present invention can be identified using the criteria set out above and can be chemically synthesized by methods known to those of skill in the art. Such methods include, but are not limited to, exclusive solid phase synthesis, partial solid phase synthesis, fragment condensation or classical solution synthesis (Merrifield (1963) *J. Am. Chem. Soc.* 85:2149; Merrifield (1986) *Science* 232:341). Further, viral PLA_2 peptides can be isolated and purified by standard purification methods including chromatography (*e.g.* ion exchange, affinity, and sizing column chromatography or high performance liquid chromatography), centrifugation, differential solubility, or by other standard techniques familiar to a worker skilled in the art. Once synthesized, the authenticity of the resulting peptides can be verified using the assay procedures disclosed herein.

Inactive Viral PLA_2s

The present invention also includes viral PLA_2 proteins, peptides or fragments thereof, the sequence of which produces an inactive protein (*i.e.* one that no longer possesses PLA_2 activity), or has been manipulated to produce an inactive protein.

Once a viral PLA_2 protein sequence has been identified using the criteria set out above, the nucleic acid sequence encoding the protein can be isolated. Standard molecular biology techniques can then be employed to alter the nucleic acid sequence and, thus the protein sequence that it encodes. Methods of altering nucleic acid sequences are well-known in the art, for example, site-directed mutagenesis or PCR-based techniques can be employed.

Naturally inactive mammalian $sPLA_2$ proteins, generated from pseudogenes, are known. An amino acid motif: HDXXY (where X = any amino acid) is found in the active site of all known $sPLA_2$ proteins (Dennis (1994) *J. Biol. Chem.* **269**:13057-13060). In the inactive $sPLA_2$ proteins, the conserved aspartic acid residue (D), subsequent to the conserved histidine residue (H) in the above motif, is frequently mutated to a basic amino acid. The HDXXY motif is also found in viral PLA_2s, therefore, one example of an inactive viral PLA_2 protein

would be one in which the aspartic acid residue in this motif is exchanged for a basic amino acid such as, lysine, arginine or histidine..

It is envisioned that such inactive viral PLA_2 proteins or peptides will retain their ability to induce a specific immune response and that they will, therefore, be useful in the development of vaccines.

Uses of Viral PLA_2s

The viral PLA_2s of the present invention can be used in manufacturing processes, in the clinical diagnosis and treatment of diseases, and in basic research settings.

1) Research

At present, purified bee venom PLA_2 is commonly used in research, for example as a reference standard when determining the PLA_2 enzyme activity contained in various preparations of isolated enzyme. The viral PLA_2 of PPV, however, has a 40 to 100-fold higher specific activity than that of bee venom PLA_2 which makes it better suited to various research applications. In addition, while some parvovirus PLA_2s demonstrate specificity for certain phospholipids (*e.g. Gm*DNV PLA_2 is specific for phosphatidylinositol), others have a broad substrate specificity thus making their use widely applicable.

2) Screening for viral PLA_2 inhibitors

The viral PLA_2s of the present invention are also useful for screening compounds in order to identify agents capable of inhibiting PLA_2 activity. Useful inhibitory agents are identified with a range of assays employing viral PLA_2s or nucleic acids encoding viral PLA_2s. As examples, protein binding assays, nucleic acid binding assays, gel shift assays, cell-based assays, mixed micelle assays, and the like are useful approaches.

In one embodiment of the present invention, there is provided a screening assay in which a candidate inhibitor is added to a solution containing a viral PLA_2 and a labelled phospholipid

substrate, and the inhibition of PLA_2 activity is then determined. In order to determine whether the inhibitory activity of the candidate inhibitor compound is specific, PLA_2 activity in the presence of the candidate inhibitor, is compared to the following: a) the PLA_2 activity in the absence of the candidate compound, b) in the presence of a well-characterized PLA_2 inhibitor, c) in the presence of a PLA_2 agonist, d) in the presence of activators and inhibitors of other phospholipase enzymes, for example PLC, and e) in the presence of both the candidate inhibitor agent and the well-characterized PLA_2 inhibitor. PLA_2 activity can be determined according to the methods described herein. Such *in vitro* screening procedures are especially useful in identifying candidate agents that can be subsequently tested *in vivo* for their ability to inhibit viral PLA_2s.

In another embodiment of the present invention, the *in vitro* assay is an automated, cost-effective, high-throughput screening protocol that can be used to survey a large number of test compounds for their ability to inhibit viral PLA_2 activity.

Potential inhibitory compounds are screened from large libraries of synthetic or natural compounds. Numerous means are currently used for random and directed synthesis of saccharide, peptide, and nucleic acid based compounds. Synthetic compound libraries are commercially available from a number of companies including Maybridge Chemical Co. (Trevillet, Cornwall, UK), Comgenex (Princeton, N.J.), Brandon Associates (Merrimack, N.H.), and Microsource (New Milford, Conn.). A rare chemical library is available from Aldrich (Milwaukee, Wis.). Combinatorial libraries are also available and can be prepared according to standard procedures. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant, and animal extracts are available from, e.g., Pan Laboratories (Bothell, Wash.) or MycoSearch (North Carolina), or are readily producible. Additionally, natural and synthetically produced libraries and compounds are readily modified through conventional chemical, physical, and biochemical means. The agent library can be naive or can be composed of structural analogs of known PLA_2 inhibitors, or can be a combination of both.

3) Gene Therapy

Viral phospholipase A_2 has been shown to be critical for the transfer of the viral genome from the late endosome to the nucleus (see Figure 7). Therefore, the present invention provides a method of improving viral-based vectors for gene therapy. An example would be the adeno-associated virus (AAV) vector currently envisioned for gene therapy. Inclusion of the viral PLA_2 gene into known gene therapy vectors can optimize the transfer of the therapeutic gene-carrying genome to the nucleus of target cells. If necessary, the viral PLA_2 gene may be modified for this purpose, for example, by site-directed mutagenesis, for optimal effectiveness and specificity.

4) Viral PLA_2-Binding Peptides

The present invention also provides for polypeptides and peptides that bind the viral PLA_2s. One exemplary method of identifying such peptide is by phage display techniques. Phage display libraries of random short peptides are commercially available, *e.g.* from New England Biolabs, Inc., which are utilized through an *in vitro* selection process known as "panning". In its simplest form, panning involves incubating the library of phage displayed peptides with a plate, or bead, coated with the target molecule, washing away unbound phage particles and finally eluting the specifically bound phage. For the purposes of the present invention, the target molecule is a viral PLA_2, or fragment thereof.

In one embodiment of the present invention, the target molecule is porcine parvovirus PLA_2 and the peptides displayed by phage display library are 12-mers.

The peptide(s) displayed by the specifically-binding phage are then isolated and sequenced by standard techniques known to those of skill in the art. In some instances the binding strength of the isolated peptide is then tested using standard techniques.

In one embodiment of the present invention, there is provided polynucleotides which encode the viral PLA_2-binding peptide. These polynucleotides can be cloned and fused with a heterologous nucleic acid. In a related embodiment, the resulting fusion gene is used to produce a fusion protein comprising the viral PLA_2-binding peptide and a heterologous protein. As discussed herein, a worker skilled in the art would readily appreciate that the

polynucleotides of the present invention can be cloned with the appropriate regulatory sequences for expression.

In one embodiment of the present invention, there is provided a fusion protein comprising a viral PLA_2-binding peptide and thioredoxin. The thioredoxin fusion protein can be used in an ELISA assay for detection of viral PLA_2s. In this assay the viral PLA_2-binding peptide of the fusion protein binds the viral PLA_2 in a sample, and the resulting complexes are detected using anti-thioredoxin antibodies. The antibodies are either labelled directly, or are detected with a secondary antibody that is labelled, for example with an enzyme in the case of ELISA assays. Such an assay is useful for detecting virus in a sample from a subject suspected of having a viral infection.

Furthermore, peptides that bind to the viral PLA_2s can be directly labelled by techniques known in the art and used as probe molecules. As such, labelled viral PLA_2-binding peptides are deemed to be within the scope of those skilled in the art in view of the teaching provided herein.

Alternatively, peptides can be selected that, by binding to the viral PLA_2, specifically inhibit the activity of the protein. The inhibitory activity of the peptides is determined using assays as known in the art and as described herein. Inhibitory viral PLA_2-binding peptides can be used therapeutically to reduce or eliminate viral infectivity. One embodiment of the present ideation provides pharmaceutical compositions comprising one or more viral PLA_2-binding peptides or polynucleotides encoding such peptides and a pharmaceutically acceptable diluent or excipient.

Anti-viral Agents

The present invention further provides a class of anti-viral agent that selectively inhibits viral PLA_2 activity. Viral PLA_2 activity is required in order for the virus to enter a host nucleus; thus, inhibition of viral PLA_2 activity blocks the ability of a virus to infect a host cell. Since

the sequence of the viral enzyme is very different from that of the host enzymes, specific inhibitors can be developed that do not impair host PLA_2 enzyme activities. The present invention provides anti-viral PLA_2 agents that inhibit viral PLA_2 activity but that are nontoxic to the host.

1) Selection of Inhibitors (Active Agents)

The anti-viral PLA_2 agents of the present invention are initially selected on the basis of the agent's capacity for inhibiting viral PLA_2 *in vitro*. Inhibition of PLA_2 activity can be determined using assays that monitor phospholipase activity including the screening assay of the present invention. This initially selected agent is then be administered to a cultured host cell population, which is subsequently exposed to a virus. The capacity of the agent to produce a detectable reduction in the infectivity and / or replication of the virus in the treated cell culture, in comparison to an untreated cell culture, is determined according to standard procedures known in the art, for example by measuring plaque forming units. Agents capable of reducing infectivity and / or replication of the virus are thereby identified as active agents.

In addition to exhibiting anti-viral activity, the anti-viral PLA_2 agents must also be selective. A selective anti-viral PLA_2 agent produces a preferential inhibition of viral PLA_2 as compared to inhibition of mammalian or host PLA_2s. Typically, the anti-viral PLA_2 agent required to produce inhibition of 50% of viral PLA_2 catalytic activity is at least one order of magnitude lower than the concentration required to produce inhibition of 50% of the catalytic activity of phospholipases other than viral PLA_2, including mammalian (host) PLA_2, PLA_1, PLC and PLD. Two exemplary methods that can be used to determine the specificity of potential anti-viral PLA_2 agents are described briefly below, although it will be appreciated by those skilled in the art that alternative methods can also be employed.

In one method PLA_2 enzyme activity is measured using a phospholipase assay familiar to one skilled in the art. An example of a suitable phospholipase assay for this procedure is described in detail in Example III. Briefly, isolated virus preparations are homogenized and centrifuged in a manner familiar to one skilled in the art of enzyme purification and supernatants are obtained. PLA_2 activity in the supernatant is then measured in the absence

and presence of various potential anti-viral PLA_2 agents. A similar procedure is used to determine the activity of mammalian or host cell PLA_2s, PLA_1s, PLCs and PLDs, in the absence and presence of the potential anti-viral agents. The selection of an anti-viral PLA_2 agent is determined by comparing the kinetic parameters of both the viral PLA_2 and the host cell PLA_2. An example of a potentially useful anti-viral PLA_2 agent for clinical trials is one that inhibits viral PLA_2 activity by at least 50 % with an IC_{50} at least one order of magnitude lower than that which inhibits the host cell PLA_2.

A second method to determine the activity of potential anti-viral PLA_2 agents is by immunofluorescence assay as described in Example VII and shown in Figure 5. A preparation of isolated virus is labelled with a fluorescent marker and then administered to a host cell culture. Entry and infection of the host cells by the virus is monitored by tracking this fluorescent tag over a period of time in the presence and absence of a potential anti-viral PLA_2 agent. The ability of the virus to infect the cell, and thus the capacity of an anti-viral PLA_2 agent to inhibit infectivity, is a function of the amount of fluorescence tag that is viewed within the cell. The higher the amount of fluorescence the weaker the inhibitory capacity of the anti-viral PLA_2 agent.

2) Antisense Oligonucleotides

The present invention contemplates antisense oligonucleotides designed to inhibit viral PLA_2 expression, wherein the antisense oligonucleotides can be complementary to all or part of the viral PLA_2 sequences, such as antisense oligonucleotides to the viral PLA_2 gene or mRNA. Such complementary antisense polynucleotides may include nucleotide substitutions, additions, deletions, or transpositions, so long as specific hybridization to the relevant target sequence is retained as a functional property of the polynucleotide. Antisense oligonucleotides include soluble antisense RNA or DNA oligonucleotides which can hybridize specifically to viral PLA_2 mRNA species and prevent transcription of the mRNA species and/or translation of the encoded polypeptide (Ching *et al.*, (1989) *Proc. Natl. Acad. Sci. U.S.A.* 86:10006; Broder *et al.*, (1990) *Ann. Int. Med.* 113:604; Loreau *et al.*, (1990) *FEBS Letters* 274:53; Holcenberg *et al.*, WO91/11535; U.S. Ser. No. 07/530,165; WO91/09865; WO91/04753; WO90/13641; and EP 386563). In one embodiment of the

present invention, the antisense oligonucleotides of the present invention exhibit little, or no, inhibition of expression of non-viral PLA_2s.

Antisense molecules are generally targeted to specific nucleic acids. "Targeting" an antisense compound to a particular nucleic acid, in the context of this invention, is a multistep process. The process usually begins with the identification of a nucleic acid sequence whose function is to be modulated. In the present invention, the target is a nucleic acid molecule encoding the viral PLA_2 gene. The targeting process also includes determination of a site or sites within this gene for the antisense interaction to occur such that the desired effect, e.g., detection or modulation of expression of the protein, will result. Within the context of the present invention, examples of intragenic sites for antisense interaction are the regions encompassing the translation initiation and the termination codon of the open reading frame (ORF) of the gene. The terms "translation initiation codon" or "start codon" can encompass several codon sequences. In the context of the invention, "start codon" and "translation initiation codon" refer to the codon, or codons, that may be present *in vivo* to initiate translation of an mRNA molecule transcribed from a gene encoding a viral PLA_2 gene, regardless of the sequence(s) of such codons.

The open reading frame (ORF) or "coding region," which is known in the art to refer to the region between the translation initiation codon and the translation termination codon, also can be targeted effectively. Other target regions include the 5' untranslated region (5'UTR) and the 3' untranslated region (3'UTR). The 5'UTR is known in the art to refer to the portion of an mRNA in the 5' direction from the translation initiation codon, and thus includes nucleotides between the 5' cap site and the translation initiation codon of an mRNA, or corresponding nucleotides on the gene. The 3'UTR is known in the art to refer to the portion of an mRNA in the 3' direction from the translation termination codon, and thus includes nucleotides between the translation termination codon and 3' end of an mRNA, or corresponding nucleotides on the gene. The 5' cap of an mRNA comprises an N^7-methylated guanosine residue joined to the 5'-most residue of the mRNA via a 5'-5' triphosphate linkage. The 5' cap region of an mRNA is considered to include the 5' cap structure itself, as well as

the first 50 nucleotides adjacent to the cap. The 5' cap region, therefore, can also be a target region for the antisense molecule.

Antisense oligonucleotides are chosen that are sufficiently complementary to the target, i.e., hybridize sufficiently well and with sufficient specificity, to give the desired effect. In the context of this invention, "hybridization" means hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleoside or nucleotide bases. Adenine and thymine, for example, are complementary nucleobases which pair through the formation of hydrogen bonds. "Complementary," as used herein, refers to the capacity for precise pairing between two nucleotides: if a nucleotide at a certain position of an oligonucleotide is capable of hydrogen bonding with a nucleotide at the same position of a DNA or RNA molecule, then the oligonucleotide and the DNA or RNA are considered to be complementary to each other at that position. The oligonucleotide and the DNA or RNA are complementary to each other when a sufficient number of corresponding positions in each molecule are occupied by nucleotides that can hydrogen bond with each other such that stable and specific binding occurs between the oligonucleotide and the DNA or RNA target. It is understood in the art that the sequence of an antisense compound need not be 100% complementary to that of its target nucleic acid to be specifically hybridizable. An antisense compound is specifically hybridizable when binding of the compound to the target DNA or RNA molecule interferes with the normal function of the target DNA or RNA to cause a loss of utility, and when there is a sufficient degree of complementarity to avoid non-specific binding of the antisense compound to non-target sequences under conditions in which specific binding is desired, i.e., under physiological conditions in the case of *in vivo* assays or therapeutic treatment, and in the case of *in vitro* assays, under conditions in which the assays are performed.

In the context of this invention, the term "oligonucleotide" refers to an oligomer or polymer of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) or mimetics thereof. This term includes oligonucleotides composed of naturally-occurring nucleobases, sugars and covalent internucleoside (backbone) linkages as well as oligonucleotides having non-naturally-occurring portions which function similarly. Such modified or substituted oligonucleotides

are often preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for nucleic acid target and increased stability in the presence of nucleases.

Examples of modified or substituted antisense compounds useful in this invention include oligonucleotides containing modified backbones or non-natural internucleoside linkages. As defined in this specification, oligonucleotides having modified backbones include those that retain a phosphorus atom in the backbone and those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, modified oligonucleotides that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides. Other oligonucleotide mimetics include those in which both the sugar and the internucleoside linkage of the nucleotide units are replaced with novel groups, while the base units are maintained for hybridization with the target nucleic acid. One such oligonucleotide mimetic that has been shown to have excellent hybridization properties is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar-backbone of an oligonucleotide is replaced with an amide-containing backbone, in particular an aminoethylglycine backbone, see for example, Nielsen *et al.*, *Science* (1991) 254:1497.

Modified oligonucleotides containing one or more substituted sugar moieties and / or one or more nucleobase substitutions are also comprehended by the present invention. The present invention also includes antisense compounds which are chimeric compounds. "Chimeric" antisense compounds or "chimeras," in the context of this invention, are antisense compounds, particularly oligonucleotides, which contain two or more chemically distinct regions, each made up of at least one monomer unit, *i.e.* one nucleotide in the case of an oligonucleotide compound. Chimeric oligonucleotides typically contain at least one region wherein the oligonucleotide is modified so as to confer upon the oligonucleotide increased resistance to nuclease degradation, increased cellular uptake, and / or increased binding affinity for the target nucleic acid.

The antisense compounds used in accordance with this invention typically comprise from about 8 to about 100 nucleobases. In one embodiment of the present invention, the antisense compounds comprise from about 8 to about 50 nucleobases. In a related embodiment, the antisense compounds comprise from about 8 to about 30 nucleobases. In another related embodiment, the antisense compounds comprise from about 15 to about 30 nucleobases. The antisense compounds can be conveniently and routinely made through the well-known technique of solid phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems (Foster City, CA). Other means for such synthesis known in the art can be additionally or alternatively employed. Similar techniques using phosphorothioates and alkylated derivatives have been employed to produce oligonucleotides.

The antisense compounds used in accordance with the present invention can be synthesized *in vitro*. The present invention also includes genetic vector constructs designed to direct the *in vivo* synthesis of antisense molecules.

3) Antibodies

Another example of anti-viral PLA_2 agents are antibodies raised against specific epitopes of viral PLA_2. Viral PLA_2s are significantly different from other PLA_2s in both their sequences and structure. Antibodies, therefore, can be raised against specific viral PLA_2 epitopes to produce PLA_2 inhibitors that are specific to viral PLA_2s and that do not interact with, or inhibit the activity of, non-viral PLA_2.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others can be immunized by injection with viral PLA_2 protein, or with any fragment or oligopeptide thereof that has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, Keyhole limpet hemolysin (KLH), and dinitrophenol. Examples of adjuvants used in humans include, BCG (bacilli Calmette-Guerin) and *Corynebacterium parvum*.

The oligopeptides, peptides, or fragments used to induce antibodies to viral PLA_2 can have an amino acid sequence consisting of as little as about 5 amino acids. In one embodiment of the present invention, amino acid sequences of at least about 10 amino acids are used. These oligopeptides, peptides, or fragments can be identical to a portion of the amino acid sequence of the natural protein that contains the entire amino acid sequence of a small, naturally occurring molecule. If required, short stretches of viral PLA_2 amino acids can be fused with those of another protein, such as KLH, and antibodies to the chimeric molecule can be produced.

Monoclonal antibodies to viral PLA_2 can be prepared using techniques that provide for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique (See, e.g., Kohler, G. *et al.* (1975) Nature 256:495-497; Kozbor, D. *et al.* (1985) J. Immunol. Methods **81**:31-42; Cote, R. J. *et al.* (1983) Proc. Natl. Acad. Sci. **80**:2026-2030; and Cole, S. P. *et al.* (1984) Mol. Cell Biol. **62**:109-120.)

For example, the monoclonal antibodies according to the invention can be obtained by immunizing animals, such as mice or rats, with purified viral PLA_2. Spleen cells isolated from the immunized animals are then immortalized using standard techniques. Those isolated immortalized cells whose culture supernatant contains an antibody that causes an inhibition of the activity of viral PLA_2 with an IC_{50} of less than 100 ng/ml are then selected and cloned using techniques that are familiar and known to one skilled in the art. The monoclonal antibodies produced by these clones are then isolated according to standard protocols.

The immortalization of the spleen cells of the immunized animals can be carried out by fusing these cells with a myeloma cell line, such as P3X63-Ag 8.653 (ATCC CRL 1580) according to the method in (1980) *J. of Imm. Meth.* 39:285-308. Other methods known to a person skilled in the art can also be used to immortalize spleen cells. In order to detect immortalized cells that produce the desired antibody against the viral PLA_2, a sample of the culture supernatant is tested using an ELISA assay for reactivity with the viral PLA_2. In order to obtain those antibodies that inhibit the enzymatic activity of viral PLA_2, the culture

supernatant of clones that produce antibodies that bind to viral PLA_2 is additionally examined for inhibition of PLA_2 activity using an appropriate assay, such as those described herein. Those clones whose culture supernatant shows the desired inhibition of viral PLA_2 activity are expanded and the antibodies produced by these clones are isolated according to known methods.

In addition, techniques developed for the production of "chimeric antibodies," such as the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used (Morrison, S. L. *et al.* (1984) *Proc. Natl. Acad. Sci.* **81**:6851-6855; Neuberger, M. S. *et al.* (1984) *Nature* **312**:604-608; and Takeda, S. *et al.* (1985) *Nature* **314**:452-454). Alternatively, techniques described for the production of single chain antibodies can be adapted, using methods known in the art, to produce viral PLA_2-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, can be generated by chain shuffling from random combinatorial immunoglobulin libraries (see for example, Burton D. R. (1991) *Proc. Natl. Acad. Sci.* **88**:10134-10137).

Antibodies can also be produced by inducing *in vivo* production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature (Orlandi, R. *et al.* (1989) *Proc. Natl. Acad. Sci.* **86**: 3833-3837; Winter, G. *et al.* (1991) *Nature* **349**:293-299).

Antibody fragments which contain specific binding sites for viral PLA_2 can also be generated. For example, such fragments include, but are not limited to, F(ab')2 fragments produced by pepsin digestion of the antibody molecule and Fab fragments generated by reducing the disulfide bridges of the F(ab')2 fragments. Alternatively, Fab expression libraries can be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity (see for example, Huse, W. D. *et al.* (1989) *Science* **246**:1275-1281).

Various immunoassays can be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using

either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between viral PLA_2 and its specific antibody. Examples of such techniques include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and fluorescence activated cell sorting (FACS). Alternatively, two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering viral PLA_2 epitopes, or a competitive binding assay can be used (see Maddox, D. E. *et al.* (1983) *J. Exp. Med.* **158**:1211-1216). These and other assays are well known in the art (see for example, Hampton, R. *et al.* (1990) *Serological Methods: A Laboratory Manual*, APS Press, St Paul, Minn., Section IV; Coligan, J. E. *et al.* (1997, and periodic supplements) *Current Protocols in Immunology*, Greene Pub. Associates and Wiley-Interscience, New York, N.Y.; Maddox, D. E. *et al.* (1983) *J. Exp. Med.* **158**:1211-1216).

Use of Anti-Viral PLA_2 Inhibitors

The identified anti-viral PLA_2 agents of the present invention can be used as commercial reagents as standards in toxicological or pharmaceutical evaluations related to PLA_2s.

The identified anti-viral PLA_2 agents of the present invention can also be used in therapeutic applications in animals and humans. The present invention describes methods of decreasing the infectivity and/or replication of a virus in a subject by administering an anti-viral PLA_2 agent to the subject. In one embodiment, the virus is a parvovirus, including mammalian parvoviruses, such as canine parvovirus (CPV), mink enteritis virus (MEV), porcine parvovirus (PPV), bovine parvovirus (BPV), goose parvovirus (GPV), feline panleukopenia virus (FPLV), and human parvoviruses, including adeno-associated virus, B19, V9 (Nguyen *et al.*, (1999) *J. Clin. Microbiol.* 37:2483), and RA-1 (Stierle *et al.*, (1987) *Ann. Rheum. Dis.* 46:219); and insect parvoviruses including densoviruses for the following hosts: *Gm Galleria mellonella*; *Ml Mythimna loreyi; Jc Junonia coenia*; *Pi Pseudoplusia includens; Ds Diatraea saccharalis; Cp Culex pipiens; Ce Casphalia extranea; Ad Acheta domesticus*; *Pf Periplaneta fuliginos;* and *Bm Bombyx mori.*

Anti-viral PLA_2 agents that selectively block viral PLA_2 activity, including chemical compounds, antisense oligonucleotides, and antibodies, can be used in the methods of the present invention.

1) Antisense Oligonucleotides

The antisense oligonucleotides of the present invention inhibit production of the viral PLA_2 polypeptides, thus preventing the virus from infecting the host. Compositions containing a therapeutically effective dosage of viral PLA_2 antisense oligonucleotides can be administered for treatment of viral infections.

Viral PLA_2 antisense compounds can be used as research reagents and diagnostics. As an example, antisense oligonucleotides, which are able to inhibit gene expression with exquisite specificity, may be used to elucidate the function of particular genes in the viral cycle. Antisense compounds can also be used to distinguish between functions of various members of viral biological pathways.

The specificity and sensitivity of antisense is also harnessed by those of skill in the art for therapeutic uses. Antisense oligonucleotides have been employed as therapeutic moieties in the treatment of disease states in animals and man. Antisense oligonucleotides have been safely and effectively administered to humans and numerous clinical trials are presently underway. It is thus established that oligonucleotides can be useful therapeutic modalities that can be configured to be useful in treatment regimes for treatment *in vitro* (*i.e.* in cell and tissue culture) and *in vivo* (*i.e.* in whole animals, especially humans).

The present invention also includes pharmaceutical compositions and formulations which include the antisense compounds of the present invention. In one embodiment of the present invention, there is provided a composition comprising one or more antisense oligonucleotides and a diluent. In an alternative embodiment the composition additionally comprises one or more compounds designed to target the antisense oligonucleotide to the affected tissue.

The pharmaceutical compositions of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including ophthalmic and to mucous membranes including vaginal and rectal delivery); pulmonary, *e.g.* by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal; intranasal; epidermal and transdermal; oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial, e.g., intrathecal or intraventricular, administration.

2) Administration of Purified or Synthetic Anti-Viral PLA_2 Inhibitors and Antibodies

The anti-viral PLA_2 agents of the present invention can be delivered alone or in combination, and can be delivered along with a pharmaceutically acceptable vehicle. Ideally, such a vehicle would enhance the stability and/or delivery properties. The invention also provides for pharmaceutical compositions containing the active factor or fragment or derivative thereof, which can be administered using a suitable vehicle such as liposomes, microparticles or microcapsules. In various embodiments of the invention, it may be useful to use such compositions to achieve sustained release of the active component.

When the anti-viral PLA_2 agents of the present invention are employed for the treatment of viral infections in a subject, they can be formulated into oral dosage forms such as tablets, capsules and the like. The compounds can be administered alone or in combination with conventional carriers, such as magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatin, tragacanth, methylcellulose, sodium carboxymethyl-cellulose, low melting wax, cocoa butter and the like. Diluents, flavoring agents, solubilizers, lubricants, suspending agents, binders, tablet-disintegrating agents and the like can also be employed. The compounds can be encapsulated with or without other carriers. In all cases, the proportion of active ingredients in said compositions both solid and liquid will be at least to impart the desired activity thereto on oral administration. The anti-viral PLA_2 agents can also be injected parenterally, in which case they are used in the form of a sterile solution containing other solutes, for example, enough saline or glucose to make the solution isotonic.

For administration by inhalation or insufflation, the anti-viral PLA_2 agents can be formulated into an aqueous or partially aqueous solution, which can then be utilized in the form of an aerosol.

For topical use, the anti-viral PLA_2 agents can be formulated in the form of dusting powders, creams or lotions in pharmaceutically acceptable vehicles, which are applied to the affected portion of the skin.

The dosage requirements of the anti-viral PLA_2 agents will vary with the particular compositions employed, the route of administration, the severity of the symptoms presented and the particular subject being treated. Such dosage requirements can be determined by standard clinical techniques, known to a worker skilled in the art. Treatment can be initiated with small dosages less than the optimum dose of the compound and thereafter increased until the optimum effect under the circumstances is reached. In general, the anti-viral PLA_2 agents of the present invention are most administered at a concentration that will afford effective results without causing any harmful or deleterious side effects. The anti-viral PLA_2 agents can be either administered as a single unit dose, or the dosage can be divided into convenient subunits administered at suitable times throughout the day.

3) Vaccines

Compositions for use as a vaccine against a virus generally comprise an immunizing amount of a viral PLA_2, or an immunogenic fragment thereof, as an antigen in a pharmaceutically acceptable vehicle. The present invention also provides for inactive viral PLA_2 proteins, peptides, or fragments thereof, which retain their ability to induce a specific immune response, for use as vaccines. For example, such vaccines can be prepared as injectables, such as liquid solutions or suspensions. Solid forms for solubilization or resuspension in a liquid before injection also can be prepared, or the preparation can be emulsified. The active or inactive antigenic capsids for vaccination can be mixed with excipients that are pharmaceutically acceptable and compatible with the active capsids. Examples of suitable excipients are water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, if desired, the vaccine can contain minor amounts of auxiliary substances such

as wetting or emulsifying agents, pH buffering agents, or adjuvants, such as aluminum hydroxide, which enhance the effectiveness of the vaccine. The vaccines are conventionally administered parenterally by injection, for example, subcutaneously or intramuscularly.

The present invention further provides for inactive recombinant live virus vaccines. Methods of generating recombinant virus particles are well-known in the art. Since viral phospholipase A_2 has been shown to be critical for the transfer of the viral genome from the late endosome to the nucleus, recombinant virus particles containing a genetically engineered PLA_2 gene encoding an inactive PLA_2 protein, will be non-infective. Such recombinant virus particles could, therefore, be combined with a pharmaceutically acceptable vehicle as described above and used to immunize a subject against subsequent infection with the active form of the virus.

The vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective and immunogenic. The quantity to be administered depends on the subject to be treated, the capacity of the subject's immune system to synthesize antibodies, and the degree of protection desired. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are peculiar to the subject. Suitable regimes for initial administration and booster shots are also variable, but are typified by an initial administration followed in one or two week intervals by a subsequent injection or other administration.

4) Gene Therapy

A viral PLA_2 peptide or biologically active fragments thereof, may also be employed in accordance with the present invention by expression of such proteins *in vivo*, which is often referred to as "gene therapy."

Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) encoding a polypeptide *ex vivo*, with the engineered cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For

example, cells may be engineered by procedures known in the art by use of a retroviral particle containing RNA encoding viral PLA_2 or a biologically active fragment thereof.

Similarly, cells may be engineered *in vivo* for expression of a polypeptide *in vivo* by, for example, procedures known in the art. As known in the art, a producer cell for producing a retroviral particle containing RNA encoding viral PLA_2, or a biologically active fragment thereof, may be administered to a patient for engineering cells *in vivo* and expression of the polypeptide *in vivo*. These and other methods for administering viral PLA_2, or a biologically active fragment thereof, by such method should be apparent to those skilled in the art from the teachings of the present invention. For example, the expression vehicle for engineering cells may be other than a retrovirus, for example, an adenovirus which may be used to engineer cells *in vivo* after combination with a suitable delivery vehicle.

Within the expression vehicle, the nucleic acid sequence encoding the polypeptide of the present invention is under the control of one or more suitable promoters. Suitable promoters which can be employed include, but are not limited to, adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoAI promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs (including the modified retroviral LTRs hereinabove described);the SV40 promoter; the ²-actin promoter; and human growth hormone promoters. Alternatively, the promoter can be the native PLA_2 gene promoter.

When the expression vehicle is a retrovirus, a retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, È-2, È-AM, PA12, T19-14X, VT-19-17-H2, ÈCRE, ÈCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, *Human Gene Therapy*, Vol. 1, pgs. 5-14 (1990). The vector can transduce the packaging cells through any means known in the art. Such means include, but

are not limited to, electroporation, the use of liposomes, and $CaPO_4$ precipitation. In one alternative, the retroviral plasmid vector can be encapsulated into a liposome, or PTH to a lipid, and then administered to a host.

The transduced producer cell line generates infectious retroviral vector particles that include the nucleic acid sequence(s) encoding the polypeptides. Such retroviral vector particles then can be employed to transduce eukaryotic cells, either *in vitro* or *in vivo*. The transduced eukaryotic cells will express the nucleic acid sequence(s) encoding the polypeptide. Eukaryotic cells which can be transduced include, but are not limited to, embryonic stem cells, embryonic carcinoma cells, hematopoietic stem cells, hepatocytes, fibroblasts, myoblasts, keratinocytes, endothelial cells, and bronchial epithelial cells.

The viral vector employed can, in one embodiment, be an adenoviral vector that includes essentially the complete adenoviral genome (Shenk *et al.*, (1984) *Curr. Topics Microbiol. Immun.* **111**(3):1-39). Alternatively, the viral vector can be a modified adenoviral vector in which at least a portion of the adenoviral genome has been deleted. The viruses used in the construction of viral vectors are generally rendered replication-defective to remove the undesirable effects of viral replication on the target cells.

The DNA sequences encoding the anti-viral PLA_2 agents employed in the present invention can be either cDNA or genomic DNA. DNA encoding either the entire anti-viral PLA_2 agent, or a portion thereof, can be used. Due to the degeneracy of the genetic code, other DNA sequences that encode substantially the same anti-viral PLA_2 agent or a functional equivalent can also be used. Multiple gene copies can also be used.

In order to produce the gene constructs of the invention, recombinant DNA and cloning methods, which are well known to those skilled in the art, can be utilized (see Sambrook *et al., Molecular Cloning, A Laboratory Manual*, 2d ed. (New York: Cold Spring Harbor Laboratory Press, 1989), including the use of restriction enzymes, site-directed mutagenesis, ligation, homologous recombination, and transfection techniques.

It will be appreciated that administration of the viral vectors of the present invention for gene therapy will be by procedures well-established in the pharmaceutical arts, *e.g.* by direct delivery to the target organ, tissue or site, intranasally, intravenously, intramuscularly, subcutaneously, intradermally and through oral administration, either alone or in combination.

The dosages administered will vary from subject to subject and will be determined by the level of decrease of infectivity and/or replication of the particular virus balanced against any risk or deleterious side effects.

The methods of the present invention will be particularly useful when the subject is infected by a particular virus. As an example, parvovirus B19 infection may lead to fifth disease in normal individuals, transient aplastic crisis in patients with underlying hemolysis, and chronic anemia due to persistent infection in immunocompromised patients. B19 infection in pregnancy can lead to hydrops fetalis and fetal loss and/or congenital infection. B19 has also been associated with inflammation and autoimmune diseases, such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), adult-onset Still's disease, and polyarthritis. It is envisioned, therefore, that the anti-viral PLA_2 agents of the present invention can be used to decrease the infectivity and/or replication of B19 in subjects with these disorders.

Improvement over Current Tools

The viral PLA_2s of the present invention are an improvement over PLA_2 standards used currently in manufacturing, clinical, and research settings. At present, bee venom is commonly used as a standard in measuring PLA_2 activity. The viral PLA_2 of PPV, however, has a 41-fold higher specific activity than bee venom. This high activity makes PPV PLA_2 ideal for use as a PLA_2 standard. The present invention overcomes the shortcomings and drawbacks often associated with treatment of viral infections, through the identification of a novel family of viral PLA_2 genes and nucleic acid sequences, amino acid sequences, clones,

vectors, antisense nucleotide sequences, and cell lines. The improvement over current tools lies in the aspects of the present invention that can be used to specifically inhibit the infectivity and / or replication of many pathogenic viruses known to cause disease in humans. The instant application describes for the first time the inhibition of a newly characterized class of phospholipase A_2 enzyme found in many viruses. Inhibition of these viral-specific enzymes is less toxic to the host since host-specific enzymes are not inhibited. This specificity is largely due to the significant differences in the sequences of viral versus non-viral PLA_2s. Thus, the present invention provides a powerful new anti-viral tool, the use of which will not affect host PLA_2s and phospholipase enzymes, but will dramatically decreases the viral PLA_2. These viral specific PLA_2s are critical components for the replication of the virus and may further be required for the pathologies associated with viral infection.

The present invention is described in further detail in the following non-limiting examples. It is to be understood that the examples described below are not meant to limit the scope of the present invention. It is expected that numerous variants will be obvious to the person skilled in the art to which the present invention pertains, without any departure from the spirit of the present invention.

EXAMPLES

Materials and Methods

Expression of VP1up fragments:

Expressed VP1ups were obtained as follows: the pBADTBX vector was constructed from the pBAD/TOPO Thiofusion expression vector (Invitrogen) by inserting a TEV protease site followed by a polylinker (*Bgl*II, *Xba*I) downstream of the thioredoxin gene. The vector contains the sequence for a His-tag downstream of this sequence. VP1up regions from porcine parvovirus [SEQ ID NO: 50], B19 human parvovirus [SEQ ID NO: 52], *Galleria mellonella* densovirus [SEQ ID NO: 54], *Bombyx mori* densovirus [SEQ ID NO: 58], *Casphalia extranea* densovirus [SEQ ID NO: 56] and adeno-associated virus [SEQ ID NO: 60] were cloned into the *Bgl*II-*Xba*I sites to obtain the respective fusion proteins. These

proteins could be purified using the His-tag on a Ni-agarose column. Subsequent cleavage by TEV protease removed the thioredoxin moiety from the fusion protein. PLA_2 activity of the fusion protein, however, was not significantly affected by the presence of thioredoxin. Usual yields were about 1 mg/litre.

Polyclonal Anti-V1up Antibodies:

Using the above expression vectors containing the V1up regions, PLA_2 was expressed and purified as described. About 250 mg of the fusion protein was injected into rabbits with adjuvants (subcutaneously and intramuscularly) according to standard protocols. Immunization was repeated after three weeks. The polyclonal antibodies that were generated were isolated by standard techniques.

Assaying PLA_2 Activity:

PLA_2 enzyme activity was established using the *E. coli* radioactive assay (Elsbach and Weiss (1991) *Methods Enzymol* 197:24). *E. coli* phospholipids were metabolically labelled for 3 hours with [^{14}C]-oleic acid (95% in *sn*-2 position). The bacteria were then autoclaved for 15min. to inactivate bacterial phospholipases, and washed. The viral sample (1-1000 pg) was added to the bacteria (10,000 cpm) in standard buffer (50 mM Tris-HCl, pH 8.0, and 10 mM $CaCl_2$) and incubated for 30min. at 37°C. The reaction was stopped by the addition of ice-cold 1% BSA. After centrifugation, 50% of the supernatant was counted.

The mixed micelles assay was also used to establish PLA_2 activity. The substrates (usually from New England Nuclear/DuPont) were 6 µM of either L-3-phosphatidylcholine,1,2-di[1-^{14}C]oleoyl (specific activity 110 mCi/mmol), L-3-phosphatidylinositol L-1-stearoyl-2-[1-^{14}C]arachidonyl (specific activity 48 mCi/mmol), or phosphatidylethanolamine L-1-palmitoyl,2-[1-^{14}C]arachidonyl (specific activity 54.6 mCi/mmol). This assay was based on that described by Manjunath *et al.*, (1994) *Biochem J.* **303**:121, with the following modifications: 1 mM Triton X-100 was used instead of deoxycholate; the reaction total volume was 50 µl and was stopped after 10 min by adding 80 µl chloroform/methanol (2:1) and 50 µl of saturated KCl solution. Separation was on silica gel thin layer chromatographic plates. Optimal separation of the phosphatidylcholine reaction products was obtained using

a solvent solution containing chloroform, methanol and water (65:35:4). For phosphatidylethanolamine and phosphatidylinositol, chloroform and methanol (87:13) were used as solvent. The separated products were quantified using a Molecular Dynamics PhosphorImager SI after drying. Bee venom PLA_2 was purchased from Sigma-Aldrich Co. (Cat. No. P9279). Only the regression lines of activity *vs.* dilution with a correlation coefficient $r^2>0.98$ were used to calculate the amount of protein to hydrolyze 50% of the substrate in the mixed micelles assay. Activity was then expressed as the μmol phospholipid hydrolyzed during the 10min. assay per ng of enzyme.

Alternatively, the first-order rate constant, k, was calculated from reaction progress curves using the integrated first-order rate equation $[P] = S_0(1-e^{-kt})$, where P is product concentration and S is substrate concentration, and in which $k = (k_{cat}/K_M)_{app}E_0$, where E_0 is the total enzyme concentration. The value of $(k_{cat}/K_M)_{app}$ represents the catalytic efficiency of the enzyme.

MIMIC Assay:

The MIMIC assay is described in Payan *et al.*, (1997) *J. Virol. Methods* 65:299 and Haberhausen *et al.*, (1998) *J. Clin. Microbiol.* **36**:628. The primers used for MIMIC PCR were (positions 836-862 in NADL-2) 5'-AGTGGGTATCGCTACTAACCTACACTC [SEQ ID NO: 47] and (positions 1207-1181 in NADL-2) 5'-GATCTGTCATCATCCAGTCTTCTATGC [SEQ ID NO: 48]. The competing MIMIC amplicon contained a deletion from positions 863-903 and was cloned into a pBluescript plasmid.

Immunofluorescence:

For immunofluorescence studies, the 3C9 monoclonal antibody (ATCC CRL-1745) was used as primary antibody (1 hour incubation). Goat anti-mouse IgG, labelled with fluorescein isothiocyanate was obtained from Sanofi Diagnostics Pasteur (Redmond, WA) and used as secondary antibody (incubation 1 hour), following standard techniques.

Confocal Immunofluorescence:

The double labelling of internalized PPV with LAMP-2 was performed using a porcine polyclonal anti-PPV antibody and the AC17 mouse anti-canine LAMP-2 monoclonal

antibody (previously reported to recognize mink LAMP-2; Hariri *et al.*, (2000) *Mol. Biol. Cell* **11**:255). Appropriate controls were performed to ensure that the antibody labelling was specific. *In situ* hybridization was performed using DIG-conjugated anti-VP1up and anti-NS1 probes, and FITC-conjugated anti-DIG antibody sandwich labelling according to the manufacturer's instructions (Roche Molecular Biochemicals). Nuclear labelling was performed by the addition of 0.02 mg/ml propidium iodide to paraformaldehyde-fixed cells after labelling. Confocal images were acquired using the 63X PlanApochromat objective of a Leica TCS SP confocal microscope equipped with argon and krypton laser sources.

Virus Inactivation:

PPV, at a concentration of about 10^{10} GE/ml, was treated with β-propiolactone (BPL) at 0.1% for 8 hours at 18°C. BPL reacts preferentially with purines, with the main targets being N-1 at adenosine, N-3 at cytidine, and N-7 at guanosine. Depurination may be an important consequence (Brown *et al.*, (1974) *J. Virol.* 14:840; Drinkwater *et al.*, (1980) *Biochemistry* 19:5087). These conditions completely inactivated the virus. The viral capsid was not affected and remained fully reactive to the different monoclonal antibodies (obtained from ATCC).

EXAMPLE I: PROTEIN SEQUENCE COMPARISONS

The parvovirus genome is depicted in Figure 1. The right-half of the parvovirus genome contains a large ORF coding for a nested set of capsid proteins (VPs) from alternative in-frame initiation codons so that these VPs differ by their *N*-terminal extensions. Initiation codons are determined by alternative splicing (eg., VP1 and VP2 of porcine parvovirus (PPV) and human parvovirus B19) or a leaky-scanning mechanism (*e.g.* VP1-4 of *Galleria mellonella* densovirus, *Gm*DNV). The VPs can be subdivided into 3 domains: common *C*-terminal domains required for capsid formation (60 copies per capsid); a small hinge-(like) domain; and unique *N*-terminal extensions. The capsid-forming domain consists of a β-barrel in which β-strands (black boxes: αA and αB are helices) are connected by loops (L1-4). These loops form most of the surface of the capsids, may contain allotropic determinants

(ats), and intertwine with neighboring subunits. Only the common domains are required to obtain stable capsids from expression vectors (Hernando *et al.*, (2000) *Virology* **267**:299; Le Gall-Recule *et al.* (1996), *J Gen Virol* **77**:2159; Miyamura *et al.*, (1994) *Proc Natl Acad Sci USA* **91**:8507; Wong *et al.*, (1994) *J Virol* **68**:4690; Brown *et al.*, (1994) *Virology* **198**:477; Martinez *et al.*, (1992) *Vaccine* **10**:684). The known 3D-structure of 4 different parvoviruses, solved by X-ray crystallography, is restricted to these domains (Simpson *et al.*, (1998) *Structure* **6**:1355; Agbandje-McKenna *et al.*, (1998) *Structure* **6**:1369; Agbandje *et al.*, (1993) *Proteins* **16**:155; Tsao *et al.*, (1991) *Science* **251**:1456). The hinge-regions for the group of parvoviruses consisting of PPV, Minute Virus of mice (MVM), mink enteritis parvovirus (MEV), canine parvovirus (CPV), and feline panleukemia parvovirus (FPV) contains a flexible polyglycine stretch that may enable the unique *N*-terminal extensions to be externalized through the 5-fold channel during maturation or early during infection (Cotmore *et al.*, (1999) *Virology* **254**:169). The *N*-terminal extensions of B19 VPs, and probably of *Gm*DNV, are permanently on the outside of the virion, and their hinge-like regions have a high content of glycine, threonine and serine. About 100 amino acids upstream of the start of VP is a conserved domain (cd). The shaded boxes in Figure 1 represent the domains (V1ups) cloned in expression vectors (VP1up: PPV-VP1up amino acids 2-174 [SEQ ID NO: 50]; B19-VP1up amino acids 2-240 [SEQ ID NO: 52]; and *Gm*DNV-VP1up amino acids 1-378 [SEQ ID NO: 54]).

Various protein sequences of the conserved domain (cd) region of the VP1up region of the largest capsid protein of 34 human, animal, and insect parvoviruses were compared by sequence alignment (Figure 2). The following parvovirus VP1ups were included in the sequence alignment: *Galleria mellonella* densovirus (*Gm* DNV) [SEQ ID NO: 1]; *Mythimna loreyi* densovirus (*Ml* DNV) [SEQ ID NO: 2]; *Junonia coenia* densovirus (*Jc* DNV) [SEQ ID NO: 3]; *Pseudoplusia includens* densovirus (*Pi* DNV)[SEQ ID NO: 4]; *Diatraea saccharalis* densovirus (*Ds* DNV) [SEQ ID NO: 5]; *Culex pipiens* densovirus (*Cp* DNV)[SEQ ID NO: 6]; *Periplaneta fuliginos* densovirus (*Pf* DNV) [SEQ ID NO: 7]; *Acheta domesticus* densovirus (*Ad* DNV)[SEQ ID NO: 8]; *Casphalia extranea* densovirus (*Ce* DNV) [SEQ ID NO: 9]; *Bombyx mori* densovirus (*Bm* DNV) [SEQ ID NO: 10]; canine parvovirus [SEQ ID NO: 11]; mink enteritis parvovirus [SEQ ID NO: 12]; mouse parvovirus 1 [SEQ

ID NO: 13]; feline panleukopenia parvovirus [SEQ ID NO: 14]; Minute Virus of Mice (MVM) [SEQ ID NO: 15]; Kilham rat parvovirus (K.Rat PV) [SEQ ID NO: 16]; porcine parvovirus [SEQ ID NO: 17]; Muscovy duck parvovirus (Mduck PV) [SEQ ID NO: 18]; goose parvovirus [SEQ ID NO: 19]; bovine parvovirus [SEQ ID NO: 20]; simian parvovirus [SEQ ID NO: 21]; chipmunk parvovirus [SEQ ID NO: 22]; Adeno-Associated Virus 2 (AAV2) [SEQ ID NO: 23]; Adeno-Associated Virus 3B (AAV3B) [SEQ ID NO: 24]; Adeno-Associated Virus 4 (AAV4) [SEQ ID NO: 25]; Adeno-Associated Virus 5 (AAV5) [SEQ ID NO: 26]; Adeno-Associated Virus 6 (AAV6) [SEQ ID NO: 27]; LuIII parvovirus (isolated from tissue culture) [SEQ ID NO: 28]; H1 parvovirus (isolated from tissue culture) [SEQ ID NO: 29]; human B19 parvovirus [SEQ ID NO: 30].

Representatives of $sPLA_2$s included in the alignment are: IA - *Naja naja* snake venom PLA_2 (amino acids 47 - 81 [SEQ ID NO: 31] and amino acids 115 - 126 [SEQ ID NO: 32]); IB - human pancreatic PLA_2 (amino acids 43 - 77 [SEQ ID NO: 33] and amino acids 116 - 127 [SEQ ID NO: 34]); IIA - human synovial fluid PLA_2 (amino acids 40 - 74 [SEQ ID NO: 35] and amino acids 106 - 117 [SEQ ID NO: 36]); IIB - gaboon viper snake venom PLA_2 (amino acids 18 - 52 [SEQ ID NO: 37] and amino acids 81 - 92 [SEQ ID NO: 38]); IIC - rat PLA_2 (amino acids 48 - 82 [SEQ ID NO: 39] and amino acids 118 - 129 [SEQ ID NO: 40]); III - Bee venom PLA_2 (amino acids 29 - 69 [SEQ ID NO: 41] and amino acids 87 - 98 [SEQ ID NO: 42]); V - human PLA2 (amino acids 40 - 74 [SEQ ID NO: 43] and amino acids 106 - 117 [SEQ ID NO: 44]); and X - human PLA_2 (amino acids 61 - 95 [SEQ ID NO: 45] and amino acids 128 - 139 [SEQ ID NO: 46]).

This analysis revealed the presence of a short stretch of high similarity, although in some cases the coding sequence was interrupted by an intron. Analysis of the 34 parvovirus sequences in this region also revealed a fully conserved HDXXY motif in all but four of the sequences (Aleutian Disease Virus and those of the Brevidensovirus genus). This HDXXY motif is also present in the catalytic site of secreted PLA_2s ($sPLA_2$s) (Figure 2).

In the $sPLA_2$ family (Dennis (1994) *J. Biol. Chem.* 269:13057-13060), the conserved H48 is assisted by D99 to polarize a catalytic water molecule that hydrolyzes the phospholipid ester. The conserved D49 and carbonyl oxygens from G30 and G32 bind a calcium ion involved

in the stabilization of the transition state. The conserved calcium-binding loop motif YXGXG is also present in VP1up, although not at the same sequence distance as in $sPLA_2$ (Fig. 2). Although $sPLA_2$s contain 5-8 disulfide bridges, which are a basis for their classification, these are absent in the parvovirus VP1up.

The comparison of VP1up sequences with those of $sPLA_2$ revealed major differences (in addition to disulfide bridges) that suggested $vPLA_2$ should be considered as a new group of the PLA_2 superfamily. The region connecting the HD helix and the D helix (Dijkstra *et al.*, (1981) *Nature* 289:604-606; White *et al.*, (1990) *Science* 250:1560-1563; Thunnissen *et al.*, (1990) *Nature* 347:689-691; Scott *et al.*, (1991) *Science* 254:1007-1010; Scott *et al.*, (1990) *Science* 250:1563-1566) was found to be minimal in $vPLA_2$ (Fig.2). Although, the VP1up from the vertebrate B19 parvovirus showed some sequence identity (e.g., NPYTH) to the group IB pancreatic phospholipase, significant homology was also observed (Fig.2) between the $vPLA_2$ and group III PLA_2 (e.g., bee venom) at the amino-terminal and in the C-terminal helices which form the hydrophobic channel for the fatty acid chain (Scott *et al.*, (1991) *Science* 254:1007-1010). The observed sequence homology would support the idea that $vPLA_2$ has a similar fold to the group III PLA_2 domain with connecting loop residues of the group I-II PLA_2 resulting in an overall different 3D structure.

Searches of the databases Prosite PS00118 and PS00119, PRINTS PR00389, Pfam PF00068 and ProDom PD000303 using standard motifs used to recognize PLA_2 failed to identify parvoviral phospholipase A_2 ($pvPLA_2$). Most probably this failure was due to the fact that these standard motifs rely heavily on the presence of cysteines which are necessary for disulfide bond formation and which are absent in $pvPLA_2$.

Therefore, the conservation of the catalytic site and the Ca^{2+}-binding loop was used to develop new motifs that allowed us to detect known and potential PLA_2s. Searches conducted using the pattern [WY]-[CIVL]-G-x-[GP]-[GKNS]-x(8,14)-D-x(2)-[AC]-x(2)-H-D-x(2)-Y, and allowing for 2 mismatches using, *e.g.* the PATTINPROT program (PBIL (http://npsa-pbil.ibcp.fr/)), or Pattern and Profile Searches (ExPASy (http://www.expasy.ch/)), identified enzymatically active $sPLA_2$s from all $sPLA_2$ groups, albeit with high background. Refined,

more specific motifs recognized subsets of $sPLA_2$, representing specific $sPLA_2$ groups, with high specificity and sensitivity. In the above motif, the one-letter notation of amino acids is used, bracketed amino acids (eg [WY]) represent alternative amino acids at that position, x represents any amino acid, x(2) represents a stretch of 2 amino acids and x(8,14) a stretch of between 8 and 14 amino acids.

For $pvPLA_2$, the specific motif is Y-x-G-P-G-x(12)-D-x(2)-A-x(2)-H-D-x(2)-Y. A search of the TrEMBL protein sequence database conducted using this motif detected all 102 $vPLA_2$ sequences, of which 5 had 1 mismatch. No additional $pvPLA_2$s were detected when 2 mismatches were allowed. Only when 3 or more mismatches were allowed were non-specific hits generated.

The general search patterns also permitted the detection of potential $sPLA_2$ and PLA_2 in other viruses. Virus infections have been reported to modify intracellular second-messenger pathways, lipid metabolism and arachidonic acid metabolism through pathways that normally are associated with PLA_2 (Abubakar *et al.* (1990) *Biochem. Biophys. Res. Commun.* **166**: 953; Shibutani *et al.*, (1997) *J. Clin. Invest.* **100**: 2054) thus indicating the importance of identifying potential PLA_2s in other viruses.

EXAMPLE II: PLA_2 ACTIVITY IN VP1up

The PLA_2 activity in the VP1up region of three divergent parvovirus genera was measured. Human B19, porcine parvovirus (PPV), and the insect parvovirus *Gm*DNV were chosen as typical representatives to demonstrate the presence of viral PLA_2 ($vPLA_2$) and to identify its role in the viral cycle.

PLA_2 activity of VP1up was established using the *E. coli* radioactive assay and the mixed micelles assay, as described above, using $sPLA_2$s from snake venom, bee venom, and pig pancreas as controls. Although the *E. coli* radioactive assay is more sensitive than the mixed micelles assay, the modified mixed micelles assay was found to be particularly reproducible. PLA_2 activity in purified virus was only detected when high concentrations of PPV virions

(>5 μg/ml) were assayed due to the fact that the PLA_2 domain resides predominantly inside the capsid at this stage. Alkali-denaturation and renaturation to disrupt the capsids and expose the VP1up increased the PLA_2 activity 50-100 fold to levels close to that of expressed VP1up. A heat-shock of 2min. at 70°C also released some VP1up as the PLA_2 activity increased about 20-50 times using this method. Polyclonal antibodies raised against PPV VP1up significantly reduced PLA_2 activity of VP1up exposed after dissociation of capsids or by heat shock, but did not affect the activity of bee venom PLA_2.

With the mixed micelles assay, expressed VP1up of PPV had a 41-fold higher PLA_2 specific activity (relative molar amounts of enzyme required to hydrolyze 50% of the substrate) than bee venom $sPLA_2$ (which had a specific activity 1360 U/mg). B19 and GmDNV VP1up were 13.5 and 88.0 times less active, respectively, than bee venom PLA_2, with detection thresholds in the low pg to ng range. The activity and pH optimum depended on the substrate. Although PPV and B19 $vPLA_2$ had similar activities at their respective pH optima (8.0 and 4.5) in the *E. coli* assay, B19 $vPLA_2$ had the same pH optimum as PPV $vPLA_2$ (pH 8.0) in the mixed micelles assay, but with a 550-fold lower activity.

The optimum Ca^{2+} concentration was similar to the extracellular (and some intracellular compartments) free Ca^{2+} concentration of about 1.2 mM (Evenas *et al.*, (1998) *Curr Opin Chem Biol* **2**:293). A residual activity of 10% at 10 μM Ca^{2+} could be measured. Addition of EDTA or EGTA (chelator of Ca^{2+}) reduced activity of PPV and B19 $vPLA_2$ to below detectable levels in the mixed micelles assay (Figure 3). No activity was observed at the resting cell cytoplasmic Ca^{2+} concentration of 0.1 μM, although this concentration may increase up to 10 μM in activated cells (Evenas *et al.*, (1998) *Curr Opin Chem Biol* **2**:293) or infected cells (Hallett *et al.*, (1982) *Biochem J.* **206**:671; Hartshorn *et al.*, (1988) *J. Immunol.* **141**:1295; Irurzun *et al.*, (1995) *J Virol* **69**:5142).

Reducing agents were not expected to influence the enzyme activity due to the absence of disulfide bridges. In fact, an increase in activity was observed in the presence of 2-mercaptoethanol (see Table 1).

Table 1. PLA_2 activity (% of activities)
(parallel *E. coli* radioactive assay used unless noted otherwise)

	B19	PPV
pH optimum (*E. coli* assay)	4.5	8
pH optimum (mixed micelles assay)	6	8
Ca^{2+}		
1 μM	9.6 %	8.6 %
1 mM	105.9 %	68.0 %
10 mM	100 %	100 %
2-Mercaptoethanol		
10 mM	148 %	189 %
100 mM	154 %	161 %
Monoclonal antibody to C-terminus of PPV VP1up (at about 0.5 μg/50 μl)[1]	100 %	6.7 %
Inhibitors[2]		
IC_{50}, manoalide, pH 8.0	3.8 ±0.4μM	2.0 ±0.3μM
IC_{50}, oleyloxyethylphosphoryl choline, pH 8.0	>20 μM	11.1 ±0.9 μM

[1]Determined by use of a mixed micelles assay.
[2]The IC_{50} is the inhibitory concentration leading to 50% inhibition.

The values of $(k_{cat}/K_M)_{app}$ for the PLA_2 activity of the expressed V1ups was also determined from the mixed micelle assays and are shown in Table 2.

Table 2. $(k_{cat}/K_M)_{app}$ Values

Phospholipase A_2 Source	$(k_{cat}/K_M)_{app}$ / M^{-1} s^{-1}
Bee venom	$(1.09\pm0.14)\times10^5$
Porcine PV V1up	$(71.9\pm9.4)\times10^5$
B19 PV V1up	$(2.5\pm0.2)\times10^4$
*Gm*DNV V1up	$(0.4\pm0.03)\times10^4$
*Bm*DNV V1up	$\sim0.5\times10^5$
*Ce*DNV V1up	$\sim0.5\times10^5$
AAV VP1up	$\sim0.75\times10^5$

Extended regions of PPV VP1up, each containing the conserved pvPLA$_2$ domain, were also expressed and their PLA$_2$ activity was determined (Figure 3B). VP1up from M1 to S174 was used throughout this work and was assigned the relative specific activity of 1.0. Sequences within this expressed peptide but outside the PLA$_2$ motif (dark-grey in the figure) contributed to the activity as shown by the relative specific activities. The activities of these extended V1up regions indicated that sequences outside the conserved domain had a large impact on the catalytic efficiency of the enzyme.

The PLA$_2$ activity of untreated virions (0.2 μg), virions after dissociation and after heat shock was also measured, using bee venom (18 ng) as a control. See Figure 3D. Samples in lanes 3, 5 and 7 were treated with anti-VP1up antibody (200 ng). This antibody reduced viral, but not bee venom, PLA$_2$ activity.

EXAMPLE III: SPECIFICITY OF PLA$_2$ ACTIVITY

The specificity of the vPLA$_2$ reaction was determined by thin layer chromatography (TLC) after hydrolysis of phosphatidylcholine substrate (PL) into fatty acid (FA) and lysophosphatidylcholine (LPL) by expressed vPLA$_2$ in the mixed micelles assay. PLA$_2$ activity was analyzed with a Molecular Dynamics PhosphorImager (%: fraction hydrolyzed in % = ((FA+LPL)/(FA+PL+LPL))x100). PLA$_2$s used in the assay were: PPV PLA$_2$(0.6 ng), B19 PLA$_2$ (250 ng), *Gm*DNV PLA$_2$ (350 ng) and bee venom PLA$_2$ (15 ng). Thioredoxin (2,000 ng) was used as a negative control. The results are shown in Figure 3A. The 3C9 monoclonal antibody, which binds to the *C*-terminus of PPV-VP1up, reduced PPV PLA$_2$ activity of VP1up to 18% of the original activity after adding 200 ng of antibody (lane 2), but not that of B19 (lane 4). Adding EGTA to a final concentration of 5 mM abolished PLA$_2$ activity (lane 5).

Following hydrolysis, TLC showed that the radioactive label, if present only in the *sn*-2 oleic acid, moved from the phospholipids to the fatty acid (free oleic acid) spots, and, when present

at both *sn*-1 and *sn*-2 positions, to both the lysophosphatidyl and fatty acid spots (Figure 3A), thus confirming the specificity of the PLA_2 activity.

The impact of Ca^{2+} concentration, pH and different substrates on the activity of $pvPLA_2$ was also investigated. Since 1 mM Ca^{2+}, 50 mM Tris-HCl, pH 8.0, and phosphatidylcholine (PC) were used in standard assays, these were set at 100% relative activity. The results of these assays are shown in Figure 3C. PPV can be seen to efficiently hydrolyse both phosphatidylethanolamine (PE) and phosphatidylinositol (PI), the alternative substrates tested.

EXAMPLE IV: INHIBITING PLA_2 ACTIVITY

PLA_2 inhibitors manoalide (MA) and oleyloxyethylphosphorylcholine (OP) inhibited the VP1up PLA_2 activity in the *E. coli* assay. The MA concentration leading to 50% inhibition of PLA_2 was 3.8±0.4 for B19 and 2.0±0.3 μM for PPV. Using OP, >20 and 11.1±0.9 μM, respectively, was required to achieve the same degree of inhibition.

EXAMPLE V: 3-D STRUCTURE OF $vPLA_2$

The 3D-structures of a large number of group I/II sPLA2 and group III sPLA2 have been solved (e.g., Renetseder *et al.*, (1985) *J. Biol. Chem.* 260:11627; Yuan and Tsai (1999) *Biochim. Biophys. Acta.* 1441:215; Dijkstra *et al.*, (1981) *Nature* 289:604); Sekar and Sundaralingam (1999) *Acta. Crystallogr. D. Biol. Crystallogr.* 55:46; van den Berg *et al.*, (1995) *Embo J.* 14:4123; Dijkstra *et al.*, (1978) *J. Mol. Biol.* 124:53). This permitted the prediction of the B19/PPV $vPLA_2$ structure, which then served to select potentially critical amino acids for site-directed mutagenesis (Figure 4). This sequence and structure alignment indicated that neither the position numbering of group I/II nor that of group III could be maintained and resulted in an adapted PPV numbering for $vPLA_2$.

EXAMPLE VI: SITE-DIRECTED MUTAGENESIS TO CREATE PLA_2 MUTANT PARVOVIRUSES

The 3-D structure of $vPLA_2$ was used to identify potentially critical amino acids in this enzyme. From this structure, residues 21 (proline), 35 (aspartic acid), 41 (histidine), 42 (aspartic acid), 63 (aspartic acid) and 88 (lysine) were selected for site-directed mutagenesis experiments.

Both the PPV-VP1up and the virus itself were submitted to site-directed mutagenesis (Weiner *et al.*, (1994) *Gene* 151:119; Kuipers *et al.*, (1990) *Prot. Eng.* 3:599). The mutants of the infectious clone of PPV (NADL-2 strain in pUC19 (Bergeron, *et al.*, (1996) *J Virol* 70:2508; Maxwell (1993) *J. Gen. Virol.* 74:1175; Brownstein, et al., (1992) *J Virol* 66:3118; Gardiner and Tattersall (1988) *J Virol* 62:1713; Corsini, *et al.*, (1996) *Adv Virus Res* 47:303; Berns (1990) *Microbiol Rev* 54:316)) were transfected into PT cells and large amounts were produced (despite their often low infectivity). Particular attention was devoted to detect possible revertants by monitoring the infectivity in two successive infection assays. The concentration of full virus particles, expressed as genome equivalents or GE per µl, was determined by a MIMIC assay. The infectivity of the suspensions was measured with the fluorescent-focus assay. The number of fluorescent nuclei in a well was counted using immunofluorescence 20 hours after infection (before fluorescent nuclei from a secondary infection could appear). The relative infectivity of the mutants was determined from the number of full particles, as established by GE in the MIMIC assay, required to obtain one fluorescent focus unit (ffu), after comparing it to the wild-type (wt) virus. Dilutions were used that gave at least 25 ffu (relative error <0.2). For wt PPV, 232 full particles were required per ffu, and this value represents 100%. The value for the relative specific infectivity of each mutant represents the result of five independent assays.

The PLA_2 activity of the wt and mutant VPIups was determined as shown in Table 3. Hydrolysis of the substrate was measured with a PhosphorImager. Different amounts of expressed pvPLA2 were assayed and the degree of hydrolysis was measured. In this assay,

0.46 ng wt PPV pvPLA$_2$ fusion protein was required to achieve 50% hydrolysis. The relative amount of each mutant required to obtain the same degree of hydrolysis yielded the relative specific activities. Only the regression lines of activity vs dilution with a correlation coefficient r^2>0.98 were used to calculate the amount of protein to hydrolyze 50% of the substrate in the mixed micelles assay.

Both the enzyme activity and viral infectivity decreased significantly when amino acids in the catalytic site (H41 and D42) were mutated (Table 3), whereas back-mutations restored the original infectivity/activity (the back-mutations served as a control to confirm the integrity of rest of genome). Among potential aspartic acids corresponding to D99 in sPLA$_2$, D63 was fully conserved in vPLA2 (Figure 2), and mutation of this residue was found to decrease strongly both infectivity and activity, indicating that the relative position of the predicted α-helices would be correct (Figure 4).

Site-directed mutagenesis of the aspartic acid residue at position 35, one of the very few amino acids that is conserved among all sPLA$_2$ and vPLA$_2$, to glutamic acid (D35E) or asparagine (D35N), had an impact that was comparable to the D63E or D63N mutations. This amino acid may be critical for the positioning of the Ca^{2+}-loop by 3 hydrogen bonds to this loop.

A surprising observation was the conservation of P21 among the vPLA$_2$ but not among the sPLA$_2$. When P21 was mutated to amino acids that occur at that position in sPLA$_2$ (R, W, L), both the infectivity and the activity were strongly reduced. It is not known whether mutations to P21 in sPLA$_2$ would increase their activities. This residue has a versatile role (Kuipers *et al.*, (1990) *Protein Eng* 3:599.) such as involvement in binding and orienting monomeric substrate, binding of the enzyme to micellar substrates and possibly shielding the catalytic site from excess water. P21 may be required in the case of vPLA$_2$ to compensate for the 3-amino acid insertion between the Ca^{2+}-binding loop and the α-helix containing H41.

K88 is conserved among parvoviruses, and in bee venom (K85) where it is involved in receptor binding (Nicolas *et al.*, (1997) *J. Biol. Chem.* **272**:7173). The potential role of this residue as a co-receptor in parvoviruses could not be established since conservative mutagenesis (K88R) in VP1up already decreased activity strongly (no receptor involved). Nevertheless, the presence of this conserved homologue of the bee venom PLA_2 receptor-binding helix is intriguing.

Table 3. Relative Specific Activity and Infectivity of pv PLA_2 Mutants

pvPLA_2 mutants (PPV)	**Position in group I/II**	**Position in group III**	**Relative specific activity (%)**	**Relative specific infectivity (%)**
wt			100	100
P21L	31	11	7.5	0.62
P21R	31	11	0.07	0.0012
P21W	31	11	4.1	0.055
D35E	42	28	0.27	0.071
D35N	42	28	0.02	0.0019
H41A	48	35	<0.007	0.0011
D42N	49	36	0.04	0.0012
HD41/42AN	48/49	35/36	<0.005	0.0011
D63A	99	64	<0.003	0.0003
D63N	99	64	0.05	0.0007
K88R	-	85	0.01	0.0035

EXAMPLE VII: IMMUNOFLUORESCENCE OF INFECTED CELLS

The observation that, upon transfection, wild-type and mutant infectious clones are both effective in producing virions indicated that $vPLA_2$ is required at some stage prior to replication, i.e. during entry. In order to assess the critical step(s), cells were infected with wt or mutant virus (P21L and P21W) at a concentration of 0.5 μg/ml and tracked by immunofluorescence. Virus was adsorbed to nonconfluent cells for 5 hours at 4°C to ensure synchronized entry when the cold medium was replaced by medium at 37°C (time-point 0 hours).

The following stages could be distinguished for wt by immunofluorescence (Figure 5): 0 hrs: strong, patchy staining on the cellular membrane; 4 hrs: virus entry with cytoplasmic, granular and perinuclear staining; 8 hrs: first nuclear staining; and 12 and 18 hrs: strong nuclear staining. Prior addition of lysosomotropic NH_4Cl reduced infection in a dose-dependent fashion (10, 20, and 50 mM resulted in 20-, 100- and 100,000-fold reduction in infectivity). At 20 mM NH_4Cl, the first nuclear staining was delayed to 18 hrs. Although the entry was fast (5-30 min), a decrease in infectivity could be obtained up to about 4 hrs post-infection by adding NH_4Cl or neutralizing antibodies suggesting a long stay in vesicular compartments. These results are consistent with the observation that parvovirus enters the cell via receptor-mediated endocytosis (Parker and Parrish (2000) *J. Virol.* **74**:1919; Basak and Turner (1992) *Virology* **186**:368; Vihinen-Ranta *et al.*, (1998) *J. Virol.* **72**:802). It has been observed (Parker and Parrish (2000) *J. Virol.* **74**:1919) that canine parvovirus (CPV) co-localizes with transferrin in perinuclear endosomes, suggesting that parvovirus infection is complex and involves multiple vesicular components. Biotinylation of virus prior to infection and detection by streptavidin-fluorescein, which only detects incoming virus, showed perinuclear staining after 4, 8, 12, 18 hrs but no nuclear staining. In contrast, immunofluorescence showed nuclear staining from about 8 hrs. It also indicated that infectivity, as measured by ffu, was reduced to about 50% by biotinylation. This perinuclear accumulation was not due to biotinylation since the same localization was obtained after inactivation of the virus without affecting the capsid (see Methods and Materials), infection, or subsequent immunofluorescence at 4, 8, 12, and 18 hrs. Although this suggests that only the viral genome is delivered from the perinuclear zone, perhaps from vesicles, into the nucleus, it remains possible that a

few virus particles entering the nucleus, which are below the detection level, are responsible for the infection.

Several differences were observed between the mutants and the wt virus. In contrast to the wt virus, the mutants showed a rather diffuse staining early during entry. Like the wt, the mutants became perinuclear after 4 hrs and could not be distinguished at this point from wt. Mutants, however, remained perinuclear for long periods (at least 18 hrs) whereas wt virus-infected cells showed a strong nuclear staining starting after 8 hrs. Occasionally, an infected nucleus was obtained after 18 hrs with the mutants. NH_4Cl exacerbated the effect of the mutations. Although the incoming virus still became perinuclear at about 4 hrs post-infection, it remained there without leading to infection. Single-stranded parvoviral genomes entering the nucleus are normally converted into double-stranded DNA by cellular DNA polymerase (P. Tattersall and S.F. Cotmore in P. Tijssen, ed., *Handbook of Parvoviruses*, vol. 1 (Boca Raton: CRC Press, 1990) 123). This dsDNA would yield productive infections as was shown with mutant or wt infectious clones. Taken together, these results indicate that PLA_2 mutants experience difficulties in transferring their genome from the perinuclear localization into the nucleus.

EXAMPLE VIII. CONFOCAL IMMUNOFLUORESCENCE OF INFECTED CELLS

The binding and entry of ^{35}S-labeled PPV were studied by confocal immunofluorescence using $4x10^5$ cells and $5x10^9$ wild type virus particles. Equivalent amounts of mutant viruses were used for the binding studies, and twice as much for the entry studies. Experiments were performed in quadruplicate. The results are shown in Figure 6A (binding) and 6B (entry). Viruses that were either bound to cells or had entered into cells were measured by a liquid scintillation method after performing washing or antibody-stripping procedures, respectively. $pvPLA_2$ mutations had no effect on virus binding to the cells or entry. Both binding and entry of virus were time- and dose-dependent (not shown).

Figure 6C shows the relative radioactivity from experiments in which 10^6cpm ^{35}S-labeled PPV particles (wt or mutant) were added to $2x10^7$ cells in Petri dishes and about 20% was recovered in the cells. After cellular fractionation, the distribution of virus over cytosolic and nuclear fractions was measured by liquid scintillation. Relative radioactivity was calculated as follows: [nuclear fraction-bound activity]/[cellular fraction-bound activity]. Standard errors were calculated from three experiments.

In addition, co-localization of PPV and LAMP-2 was studied by confocal microscopy. Anti-PPV antibodies were labeled with FITC, giving green fluorescence, and anti-LAMP-2 antibodies with Texas Red, giving red fluorescence. Co-localization of PPV and LAMP-2 resulted in yellow staining. The results are shown in Figure 6D. Both wt and mutant viruses, as well as capsids lacking PLA_2, showed extensive co-localization with LAMP-2 in the late endosomes/lysosomes.

Taken together the above results indicate that parvoviruses do not require $vPLA_2$ activity for binding to the cell surface or initial stages of entry.

EXAMPLE IX. IN SITU *HYBRIDIZATION OF INFECTED CELLS*

Figure 7 shows the distribution of viral DNA in infected cells. Incoming viral DNA was detected in the cytoplasm by *in situ* hybridization 4 (A) and 8 (B) hours post-infection (p.i.), whereas replicating DNA in the nucleus was found at 12 hours p.i. (C). At 12 hours p.i., no DNA could be detected in the nucleus in the case of the HD (D) and $P21^{PV}W$ mutants. Aphidicoline, a DNA polymerase inhibitor used at 2 μg/ml, also prevented the appearance of viral DNA in the nucleus (F). The $sPLA_2$ inhibitors tetracain (TC, at 100 μM), and oleyloxyethyl phosphorylcholine (OP, at 20 μM), also reduced the number of nuclei containing viral DNA (G).

EXAMPLE X. IDENTIFICATION OF VIRAL VLA_2-BINDING PEPTIDES

Four peptides that specifically bind to the PLA_2-containing VP1up of PPV using a phage display library. The Ph.D.™ Phage Display Peptide Library kit was obtained from New England Biolabs and used according to the manufacturer's instructions, with the exception that Linbro/Titertek (Cat. No. 76-231-05) plastic plates were used in the panning procedure to avoid high background.

The sequences peptides displayed on the phage that specifically bound to PLA_2 (p6, p7, p8 and p10) are shown in Figure 13. The nucleic acid sequences encoding these 12 amino acid peptides were subsequently cloned downstream of thioredoxin in the pBADTBX vector using the *Bgl*II and *Xba*I cloning sites, and expressed as fusion proteins.

The fusion proteins were used in ELISA assays to determine their ability to bind the PLA_2-containing VP1up of PPV. The titers obtained in ELISA with these fusion proteins and using anti-thioredoxin antibodies as secondary antibody were all, except for p10, about 3000. P10 was about 5x weaker.

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. An isolated viral polypeptide that has phospholipase A_2 activity.

2. The isolated viral polypeptide according to Claim 1, wherein said polypeptide comprises an amino acid motif:
[WY]-[CIVL]-G-x-[GP]-[GKNS]-x(8,14)-D-x(2)-[AC]-x(2)-H-D-x(2)-Y
wherein:
[] indicates the presence of one of the enclosed amino acids at this position;
x is any amino acid;
x(2) represents a stretch of 2 amino acids;
x(8,14) represents a stretch of between 8 and 14 amino acids.

3. The isolated polypeptide according to Claim 2 wherein said amino acid motif is:
Y-x-G-P-G-x(12)-D-x(2)-A-x(2)-H-D-x(2)-Y
wherein:
x(12) represents a stretch of 12 amino acids; and
wherein one of the specified amino acids Y, G, P, G, D, A, D, or Y may be exchanged singly for any other amino acid.

4. The polypeptide according to any one of Claims 1 - 3 wherein said viral polypeptide is a parvoviral polypeptide.

5. The polypeptide according to any one of Claims 1 - 3 wherein said parvoviral polypeptide is derived from *Galleria mellonella* densovirus, *Mythimna loreyi* densovirus, *Junonia coenia* densovirus, *Pseudoplusia includens* densovirus, *Diatraea saccharalis* densovirus, *Culex pipiens* densovirus, *Periplaneta fuliginos* densovirus, *Acheta domesticus* densovirus, *Casphalia extranea* densovirus, *Bombyx mori* densovirus, canine parvovirus, mink enteritis parvovirus, mouse parvovirus 1, feline panleukopenia parvovirus, Minute Virus of Mice, Kilham rat parvovirus, porcine parvovirus, Muscovy duck

parvovirus, goose parvovirus, bovine parvovirus, simian parvovirus, chipmunk parvovirus, Adeno-Associated Virus 2, Adeno-Associated Virus 3B, Adeno-Associated Virus 4, Adeno-Associated Virus 5, Adeno-Associated Virus 6, LuIII parvovirus, H1 parvovirus, or human B19 parvovirus.

6. The polypeptide according to Claim 5 wherein the parvovirus is human B19, porcine parvovirus, *Galleria mellonella* densovirus, *Casphalia extranea* densovirus, *Bombyx mori* densovirus or Adeno-Associated Virus.

7. An isolated viral polypeptide comprising an amino acid sequence as set out in SEQ ID NO: 55; SEQ ID NO: 57 or SEQ ID NO: 59.

8. An isolated viral polypeptide that has phospholipase A_2 activity and comprising an amino acid sequence as set out in SEQ ID NO:49; SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57 or SEQ ID NO:59.

9. The polypeptide according to any one of Claims 1- 8 wherein said polypeptide is chemically synthesized.

10. The polypeptide according to any one of Claims 1-9 wherein said polypeptide fused to a heterologous polypeptide.

11. A polypeptide that is an inactive variant of a viral polypeptides wherein said viral polypeptide comprises an amino acid motif:
[WY]-[CIVL]-G-x-[GP]-[GKNS]-x(8,14)-D-x(2)-[AC]-x(2)-H-D-x(2)-Y
wherein:
[] indicates the presence of one of the enclosed amino acids at this position;
x is any amino acid;
x(2) represents a stretch of 2 amino acids;
x(8,14) represents a stretch of between 8 and 14 amino acids.

12. An isolated polynucleotide encoding a viral polypeptide that has phospholipase A_2 activity.

13. The isolated polynucleotide according to Claim 13, wherein said polypeptide comprises an amino acid motif:
[WY]-[CIVL]-G-x-[GP]-[GKNS]-x(8,14)-D-x(2)-[AC]-x(2)-H-D-x(2)-Y
wherein:
[] indicates the presence of one of the enclosed amino acids at this position;
x is any amino acid;
x(2) represents a stretch of 2 amino acids;
x(8,14) represents a stretch of between 8 and 14 amino acids.

14. The isolated polynucleotide according to Claim 14 wherein said amino acid motif is:
Y-x-G-P-G-x(12)-D-x(2)-A-x(2)-H-D-x(2)-Y
wherein:
x(12) represents a stretch of 12 amino acids; and
wherein one of the specified amino acids Y, G, P, G, D, A, D, or Y may be exchanged singly for any other amino acid.

15. The isolated polynucleotide according to any one of Claims 12 - 14, wherein said polynucleotide is DNA, cDNA, RNA or genomic DNA.

16. The isolated polynucleotide according to anyone of Claims 12 - 15, wherein said polynucleotide is double stranded or single stranded.

17. The isolated polynucleotide according to any one of Claims 12 - 16, wherein said polynucleotide is fused to a heterologous polynucleotide.

18. The isolated polypeptide according to Claim 17 wherein said heterologous polynucleotide encodes a heterologous polypeptide.

19. An isolated polynucleotide comprising a nucleic acid sequence as set out in SEQ ID NO:56; SEQ ID NO:58 or SEQ ID NO:60.

20. An isolated polynucleotide encoding a viral polypeptide that has phospholipase A_2 activity and comprising a nucleic acid sequence as set out in SEQ ID NO:50; SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58 or SEQ ID NO:60.

21. An antisense oligonucleotide complementary to the polynucleotide according to any one of Claims 12 - 16, 19 or 20.

22. Use of the antisense oligonucleotide according to Claim 21 to inhibit viral replication and / or infectivity in a subject.

23. A vector comprising the isolated polynucleotide according to any one of Claims 12 - 20.

24. The vector according to Claim 23 wherein said polynucleotide is operatively linked to one or more regulatory sequences.

25. A method of producing a host cell comprising genetically engineering cells with the vector according to Claim 23 or 24.

26. The host cell produced according to Claim 25.

27. A host cell comprising the vector according to Claim 23 or 24.

28. A host cell comprising the isolated polynucleotide according to any one of Claims 12 - 20.

29. A method of producing a recombinant virus comprising genetically engineering viral particles with the polynucleotide, or fragment thereof, according to Claims 12 - 16, 19 or 20, or with the vector according to Claim 23 or 24.

30. The recombinant virus produced according to Claim 29.

31. An antibody that specifically binds to the polypeptide of any one of Claims 1-9.

32. The antibody according to Claim 17 or 18, wherein said antibody is polyclonal.

33. The antibody according to Claim 17 or 18, wherein said antibody is monoclonal.

34. Use of a polypeptide according to any one of Claims 1- 9 as an *in vitro* standard for measuring phospholipase A_2 activity.

35. A method of screening compounds in order to identify an agent that inhibits viral phospholipase A_2 activity, comprising the steps of:

i) incubating a viral phospholipase A_2 and a phospholipid substrate with a candidate agent under conditions whereby, but for the presence of the agent said viral phospholipase would hydrolyse said phospholipid, and

ii) measuring the inhibition of viral phospholipase A_2 activity.

36. An anti-viral agent that selectively inhibits the activity of a viral phospholipase isolated according to the method of Claim 35.

37. The anti-viral agent according to Claim 36, wherein said anti-viral agent is capable of producing a detectable decrease in infectivity and / or replication of a virus.

38. The anti-viral agent according to Claim 36 or 37, wherein said anti-viral agent is an antisense oligonucleotide that binds specifically to mRNA encoding said viral phospholipase.

39. The anti-viral agent according to Claim 38 wherein said antisense oligonucleotide is about 8 to about 100 nucleobases.

40. The anti-viral agent according to Claim 39 wherein said antisense oligonucleotide is about 8 to about 50 nucleobases.

41. The anti-viral agent according to Claim 40 wherein said antisense oligonucleotide is about 15 to about 30 nucleobases.

42. The anti-viral agent according to Claim 36 or 37, wherein said anti-viral agent is an antibody wherein said antibody is monoclonal or polyclonal.

43. A method of identifying an anti-viral agent that selectively inhibits the activity of a viral phospholipase A_2, comprising:

i) selecting a candidate molecule on the basis of the capacity of the candidate molecule to inhibit viral phospholipase A_2 in an *in vitro* assay employing a predetermined amount of a standardized preparation of viral phospholipase A_2;

ii) treating a cultured host cell population with the candidate molecule;

iii) exposing the host cell population to a virus;

iv) determining the reduction in the infectivity and / or replication of the virus;

v) determining the growth of the host cell population; and

vi) comparing the growth to the growth of an untreated cultured host cell population that has been exposed to a virus;

wherein a reduction in the infectivity and / or replication of the virus and increased growth of the host cell population in comparison to the untreated

host cell population is indicative of selective inhibition of the viral phospholipase A_2.

44. An anti-viral agent that selectively inhibits the activity of a viral phospholipase isolated according to the method of Claim 43.

45. The anti-viral agent according to Claim 44, wherein said anti-viral agent is capable of producing a detectable decrease in infectivity and / or replication of a virus *in vivo*.

46. The anti-viral agent according to Claim 44 or 45, wherein said anti-viral agent is an antisense oligonucleotide.

47. The anti-viral agent according to Claim 44 or 45, wherein said anti-viral agent is a monoclonal antibody.

48. Use of the anti-viral agent according to any one of Claims 36 - 42 or 44 - 47 to decrease the infectivity and / or replication of a virus in a subject.

49. The use according to Claim 48 wherein said virus is a parvovirus.

50. Use of the anti-viral agent according to any one of Claims 36 - 42 or 44 - 47 to inhibit a viral phospholipase A_2 in a subject in need of such therapy.

51. The use according to Claim 50 wherein said subject has a viral-associated disease or disorder.

52. The use according to Claim 51 wherein said viral-associated disease is associated with a parvovirus.

53. The use according to Claim 52 wherein said viral-associated disease is rheumatoid arthritis, systemic erythematosus, adult-onset Still's disease or polyarthritis.

54. Use of the anti-viral agent according to any one of Claims 36 - 42 or 44 - 47 to manufacture a medicament to decrease the infectivity and / or replication of a virus in a subject.

55. Use of the anti-viral agent according to any one of Claims 36 - 42 or 44 - 47 to manufacture a medicament to treat a viral-associated disease.

56. Use of the antisense oligonucleotide according to any one of Claims 38 - 41 to manufacture a medicament to decrease the infectivity and / or replication of a virus in a subject.

57. Use of the antisense oligonucleotide according to any one of Claims 38 - 41 to manufacture a medicament to treat a viral-associated disease.

58. Use of the isolated polynucleotide according to any one of Claims 12 - 20 for gene therapy.

59. Use of the isolated polynucleotide according to any one of Claims 12 - 20 to improve a gene therapy vector.

60. The use according to Claim 59 wherein said gene therapy vector is based on an Adeno-Associated virus.

61. A peptide that specifically binds a viral phospholipase A_2.

62. The peptide according to Claim 61 wherein said peptide inhibits the viral phospholipase A_2.

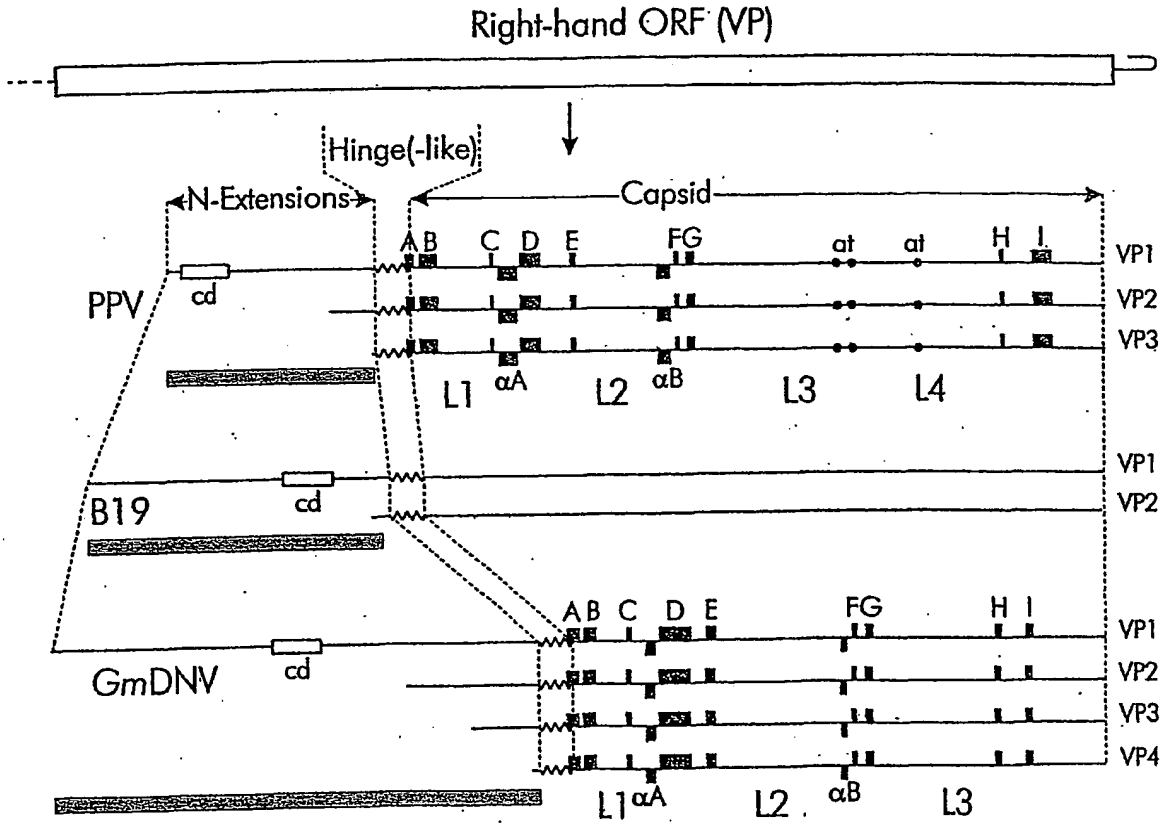
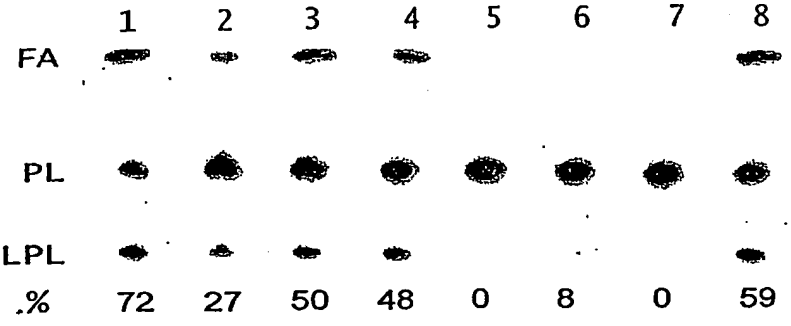

Right-hand ORF (VP)
Hinge(-like)
N-Extensions
Capsid
A B C D E FG at at H I
PPV
cd
VP1
VP2
VP3
L1 αA L2 αB L3 L4
B19
cd
VP1
VP2
GmDNV
cd
A B C D E FG H I
VP1
VP2
VP3
VP4
L1 αA L2 αB L3

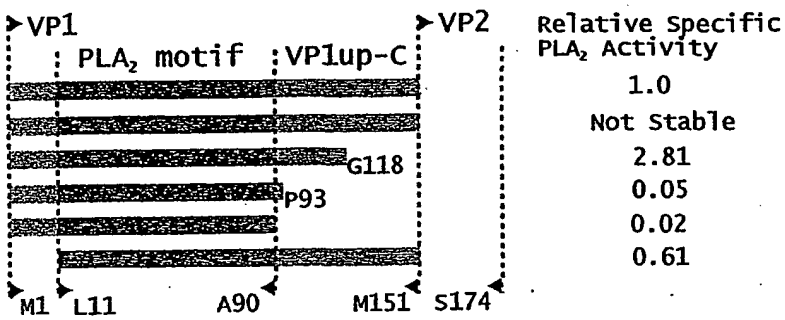
Figure 1

Figure 2

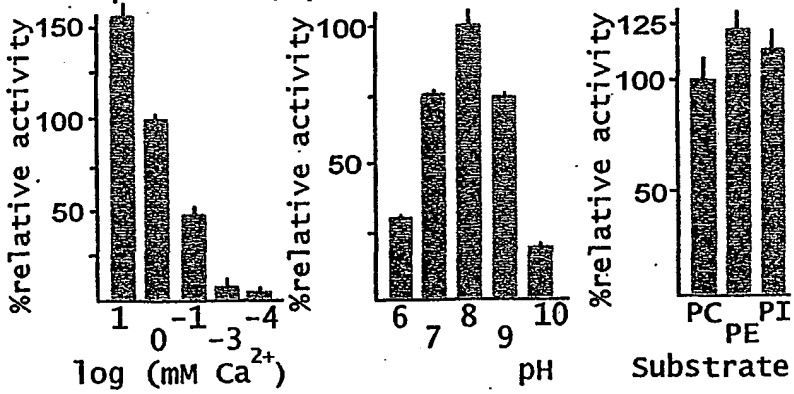
A. Activity of expressed VP1up PLA_2



B. Size of PPV VP1up and PLA_2 activity



C. Impact Ca^{2+}, pH and substrate on $pvPLA_2$



D. PLA_2 activity of PPV virions

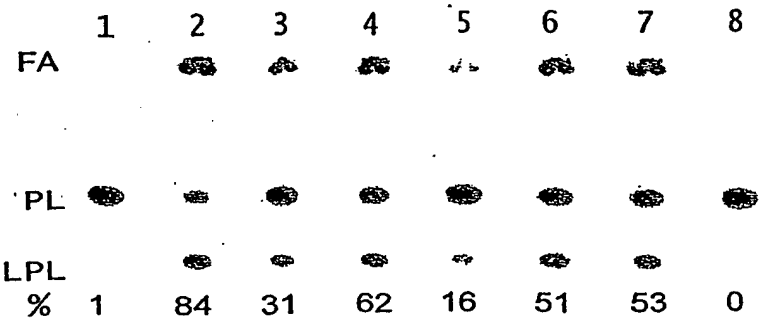


Figure 3

```
                20          30                    HHHHHHHHHHHHHHHHHH 60         70          80          HHHHHHHHHHHHHHHHHH .          120
I/II: 18:PFLEYNNYGCYCGLGGS------GTPVDELDKCCQTHDNCYDQAKKLDSCKFLLDNPYTHTYSYSCSGSAITCSSKNKECEAFICNCDRNAAICFSKAPYNKAHKNLDTKKYCQS     :126
B19 :120:QVSVQLPGTNYVGPGNELQA---GPPQSAVDSAARIHDFRYSQLAKLGI------NPYTH---------------------WTVADEELLKNIKNETGFQAQVVKDYFTLKGAAAP :205
PPV :  8:ARGLTLPGYKYLGPGNSLDQ---GEPTNPSDAAAKEHDEAYDKYIKSGK------NPYFY---------------------FSAADEKFIKETEHAKDYGGICIGHYFFRAKRAFAP : 93
III :  1:   IIYPGTLWCGHGNKSSGPNELGRFKHTDACCRTHDMCP--------------------DVMSAGESKHGLTNTASHTRLSCDCDDKFYDCLKNSA----DTISSYFVGKMYFNL  : 90
                          10          20    HHHHHHHHHHH                          40          50          60HHHHHHHHHHHH            HHHHHHHHHHHHH
```

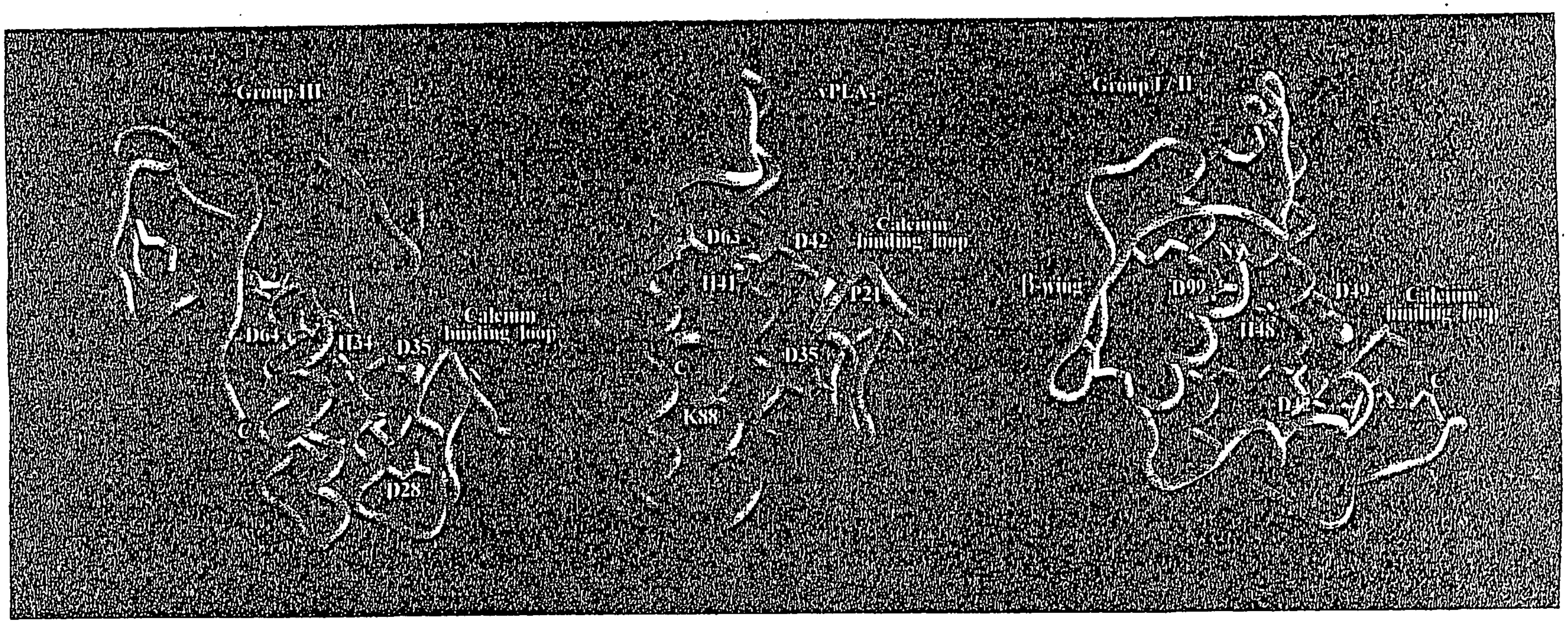


Figure 4

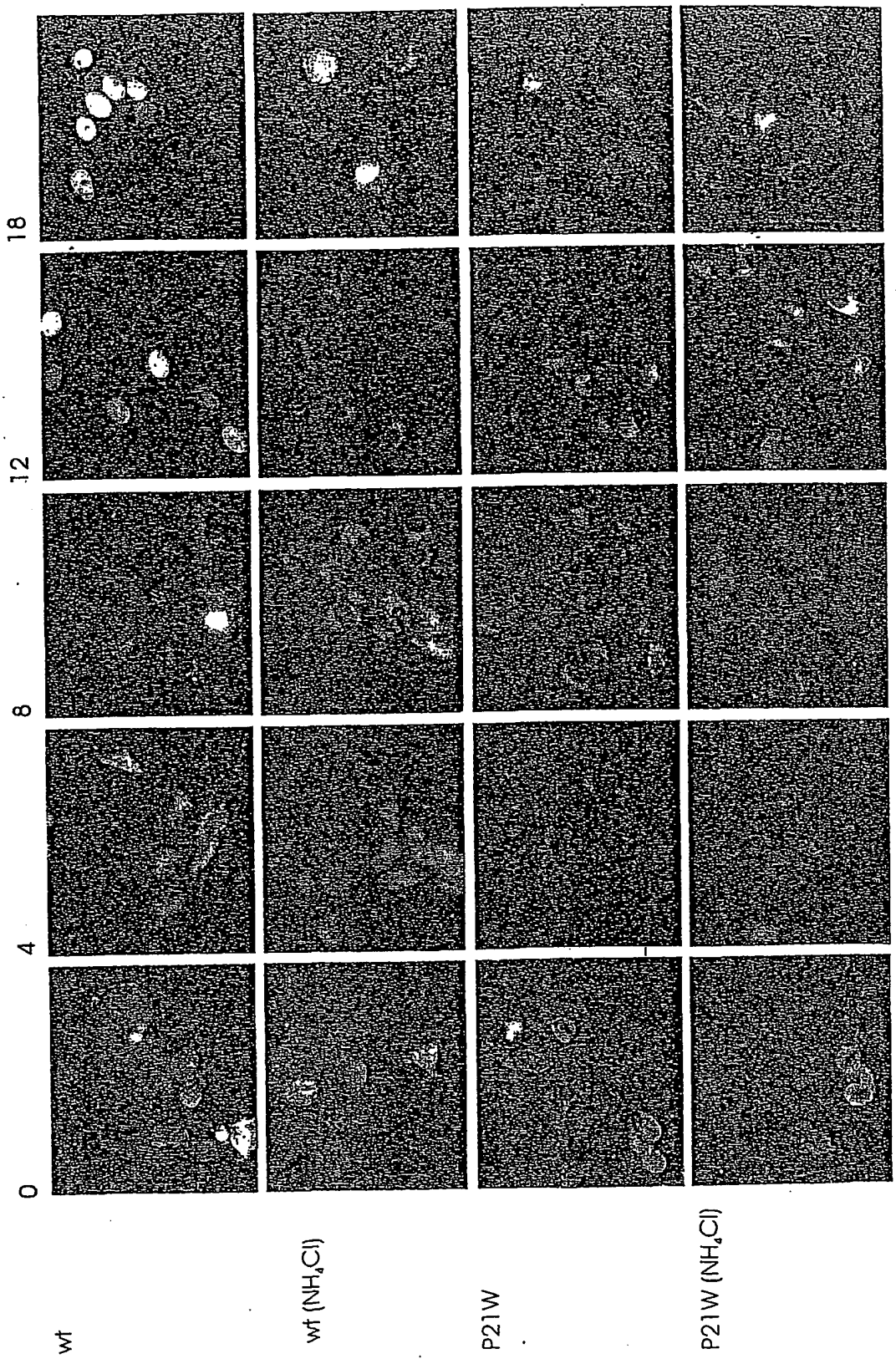
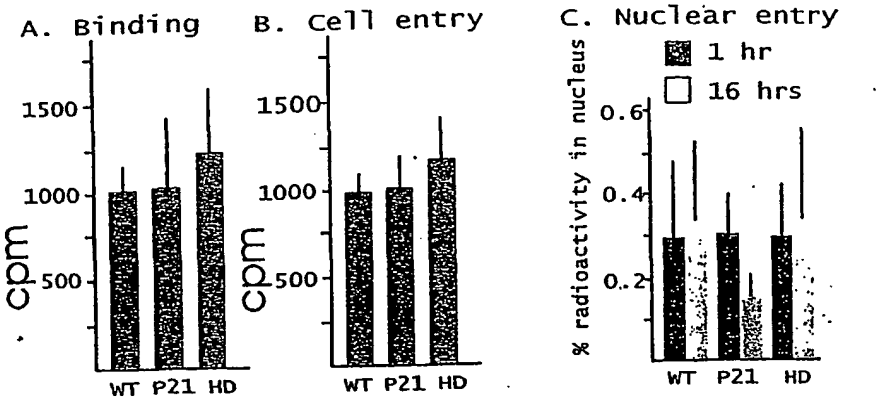


Figure 5


A. Binding
B. Cell entry
C. Nuclear entry
1 hr
16 hrs
cpm
1500
1000
500
% radioactivity in nucleus
0.6
0.4
0.2
WT P21 HD
WT P21 HD
WT P21 HD

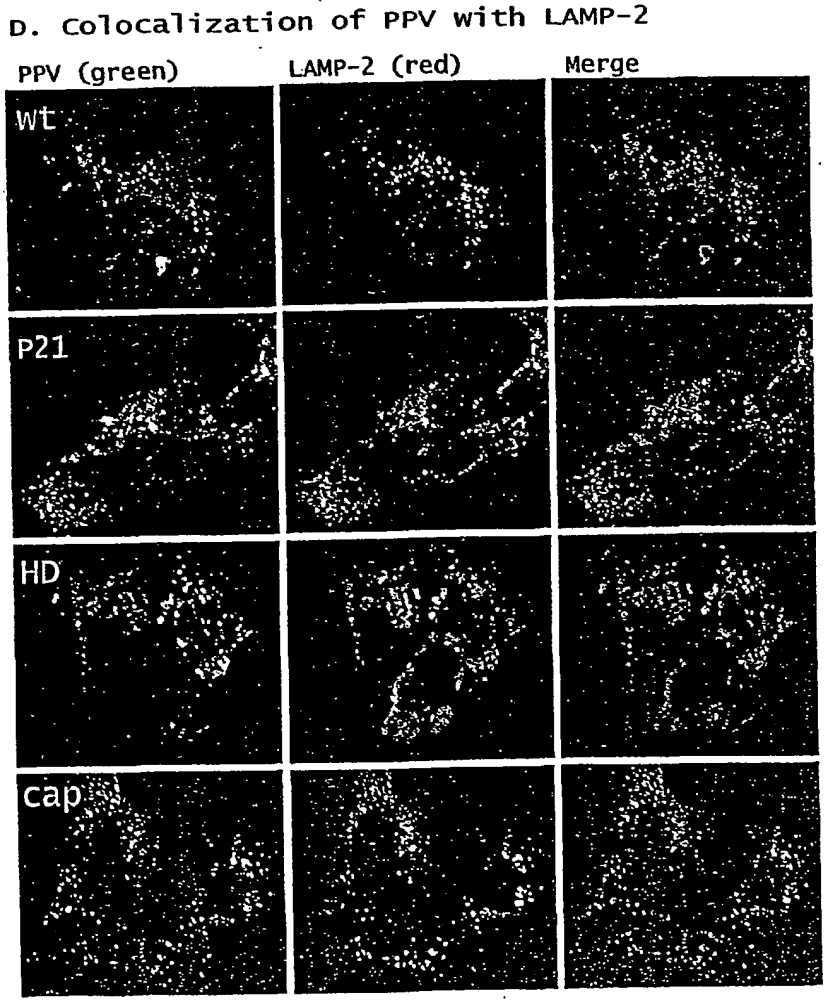

D. Colocalization of PPV with LAMP-2
PPV (green)
LAMP-2 (red)
Merge
wt
P21
HD
cap

Figure 6

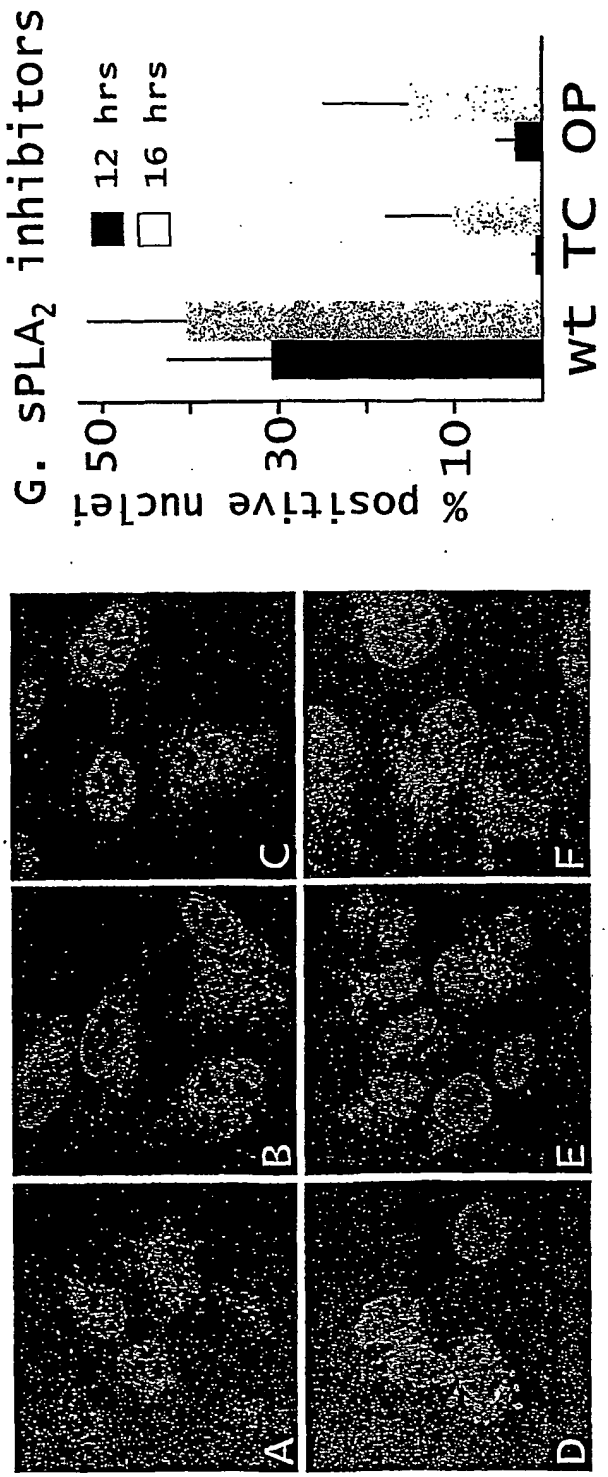


Figure 7

Porcine Parvovirus V1up

```
  1 GCGCCTCCTGCAAAAAGAGCAAGAGGACTAACTCTACCAGGATACAAATA
 51 CCTTGGTCCAGGAAACTCACTAGACCAAGGAGAACCAACTAATCCATCAG
101 ACGCCGCAGCAAAAGAACACGACGAAGCCTACGACAAATACATAAAATCT
151 GGAAAAAATCCATACTTCTACTTCTCAGCAGCTGATGAAAAATTCATAAA
201 AGAAACTGAACACGCAAAAGACTACGGAGGTAAAATTGGACATTACTTCT
251 TCAGAGCAAAGCGTGCCTTTGCTCCAAAACTCTCAGAAACAGACTCACCA
301 ACTACATCTCAACAACCAGAGGTAAGAAGATCGCCGAGAAAACACCCAGG
351 GTCTAAACCACCAGGAAAAAGACCTGCTCCAAGACATATTTTTATAAACT
401 TAGCTAAAAAAAAAAGCTAAAGGGACATCTAATACAAACTCTAACTCAATG
451 AGTGAAAATGTGGAACAACACAACCCTATTAATGCAGGCACTGAATTGTC
501 TGCAACAGGAAATGAA
```

B19 Parvovoirus V1up

```
  1 AGTAAAGAAAGTGGCAAATGGTGGGAAAGTGATGATAAATTTGCTAAAGC
 51 TGTGTATCAGCAATTTGTGGAATTTTATAAAAAGGTTACTGGAACAGACT
101 TAGAGCTTATTCAAATATTAAAAGATCATTATAATATTTCTTTAGATAAT
151 CCCCTAGAAAACCCATCCTCTCTGTTTGACTTAGTTGCTCGTATTAAAAA
201 TAACCTTAAAAACTCTCCAGACTTATATAGTCATCATTTTCAAAGTCATG
251 GACAGTTATCTGACCACCCCCATGCCTTATCATCCAGTAGCAGTCATGCA
301 GAACCTAGAGGAGAAAATGCAGTATTATCTAGTGAAGACT ACACAAGCC
351 TGGGCAAGTTAGCGTACAACTACCCGGTACTAACTATGTTGGGCCTGGCA
401 ATGAGCTACAAGCTGGGCCCCCGCAAAGTGCTGTTGACAGTGCTGCAAGG
451 ATTCATGACTTTAGGTATAGCCAACTGGCTAAGTTGGGAATAAATCCATA
501 TACTCATTGGACTGTAGCAGATGAAGAGCTTTTAAAAAATATAAAAAATG
551 AAACTGGGTTTCAAGCACAAGTAGTAAAAGACTACTTTACTTTAAAAGGT
601 GCAGCTGCCCCTGTGGCCCATTTTCAAGGAAGTTTGCCGGAAGTTCCCGC
651 TTACAACGCCTCAGAAAAATACCCAAGC
```

***Galleria mellonella* Densovirus V1up**

```
  1 ATGTCTTTCTTCAAAAATCAGTTGATACATCGCGCACGACCTGGTTATCG
 51 TATAATACCGGAAAGTACTGTTACTGAAGATATTGAATTAGGTACTATTG
101 GTGAAGAAACTCCATTGTTAAGTGAAGGTGTTATTACAGCTGTAGAAGAA
151 GGTGCTATTGGATTACCAGAAGTTGCTATTGGTGTGGCTGGTGCTATTGG
201 AACACATGCACATGAATGGTGGAGAGATAGATACGCTTTTAAATCTGTTT
251 TAACTGGTAATTATACTGATTTAAAAGGAAATCCTTTAAAACCTAGAAAC
301 GCTATTCCTGAAAAAATTAAACAACTAGGAAAGAAAATATTTCAAGGAGA
351 TTTTAATCGTGCATTTCCTGATAATTTAAAATTGGAAACTGAAAAAGAAA
401 AAGCTGATTTATTAAGATATTATAATCATAATAGAAGATTAGCTGGACTA
451 AGTGAAGCTTATCCACAAGGGAAAGGATATGCTTATGCTAAAAGTCAAAA
501 AGTATTAGAAGCTGAACGACGTGGATTAACTGTTCCTGGATATAAATATC
551 TTGGTCCTGGAAATTCATTGAATAGAGGTCAACCTATAAATCAAATAGAC
601 GAAGACGCTAAAGAACACGACGAAGCTTATGATAAAGTGAAAACAAGTCA
651 AGAAGTAAGTCGAGCAGATAATACATTTGTTAATAAAGCGTTAGATCACG
701 TGGTTAATGCTATTAATTTTAAAGAAACACCTGGTAACGCTTTTGGTGCT
751 GCTATTGGAGCTATTGGAATTGGAACTAAGCAAGCTATTGAAAAATATAG
801 TGGAGTAATCTACCCTTCTGTTTCAGGT
```

Figure 8

***Bombyx mori* Densovirus V1up**

```
  1 ATGCCTCGTATTCATTTTCCTTATCATAATTATCTTGGTCCGGGTACTGA
 51 TAACTTTGAAAAAAATCCAATAGACGAAGACGACGCTATCGCGAGGTCGC
101 ATGATTTGGCTTACGATAAAGTAACCAATCATAAGGAAGTTTTTCAAGCT
151 GATAAACAGGCCCGTGACGAGTTTTTTACTTCATTTGTGCATACTGGAAA
201 CGTGCATAGTTTAATTGGCGGTATTGGACTTGGAACTAAAAATTTGGTAG
251 AAGAACATGTACTAGGTAAACCCTTGTACGGA
```

***Casphalia extranea* Densovirus V1up**

```
  1 ATGCCTCGTATTCATTTTCCTTATCATAATTATCTTGGTCCGGGCAGTGA
 51 TAACTTTAAAAAAACAACCAGTAGACGAAGACGACGCAATAGCCAGAGCAC
101 ATGACCTGGATTACGATAAAGCAAGCTCTGATAAAGACATTTTCAAGGCT
151 GATAAGCAGGCTCGCGACGAGTTTTTCAGTTCATTTGTGCACAGCGGAAA
201 CTTGCATAGTTTAATTGGTGGACTAGGACTTGGAGCTAAAAATCTAGTAG
251 AAGAGCATGTACTAGGTAAGTCCTTGTACGGT
```

Adeno-Associated Virus V1up

```
  1 ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTCTGA
 51 AGGAATAAGACAGTGGTGGAAGCTCAAACCTGGCCCACCACCACCAAAGC
101 CCGCAGAGCGGCATAAGGACGACAGCAGGGGTCTTGTGCTTCCTGGGTAC
151 AAGTACCTCGGACCCTTCAACGGACTCGACAAGGGAGAGCCGGTCAACGA
201 GGCAGACGCCGCGGCCCTCGAGCACGACAAAGCCTACGACCGGCAGCTCG
251 ACAGCGGAGACAACCCGTACCTCAAGTACAACCACGCCGACGCGGAGTTT
301 CAGGAGCGCCTTAAAGAAGATACGTCTTTTGGGGGCAACCTCGGACGAGC
351 AGTCTTCCAGGCGAAAAAGAGGGTTCTTGAACCTCTGGGCCTGGTTGAGG
401 AACCTGTTAAGACGGCTCCGGGAAAAAAGAGGCCGGTAGAGCACTCTCCT
451 GTGGAGCCAGACTCCTCCTCGGGAACCGGAAAGGCGGGCCAGCAGCCTGC
501 AAGAAAAAGATTGAATTTTGGTCAGACTGGAGACGCAGACTCAGTACCTG
551 ACCCCCAGCCTCTCGGACAGCCACCAGCAGCCCCCTCTGGTCTGGGAACT
601 AATACG
```

Figure 8 (con.)

Porcine Parvovirus V1up

1 APPAKRARGLTLPGYKYLGPGNSLDQGEPTNPSDAAAKEHDEAYDKYIKS
51 GKNPYFYFSAADEKFIKETEHAKDYGGKIGHYFFRAKRAFAPKLSETDSP
101 TTSQQPEVRRSPRKHPGSKPPGKRPAPRHIFINLAKKKAKGTSNTNSNSM
151 SENVEQHNPINAGTELSATGNE

B19 Parvovirus V1up

1 SSKESGKWWESDDKFAKAVYQQFVEFYKKVTGTDLELIQILKDHYNISLD
51 NPLENPSSLFDLVARIKNNLKNSPDLYSHHFQSHGQLSDHPHALSSSSSH
101 AEPRGENAVLSSEDLHKPGQVSVQLPGTNYVGPGNELQAGPPQSAVDSAA
151 RIHDFRYSQLAKLGINPYTHWTVADEELLKNIKNETGFQAQVVKDYFTLK
201 GAAAPVAHFQGSLPEVPAYNASEKYPSAYNASEKYPS

***Galleria mellonella* Densovirus V1up**

1 MSFFKNQLIHRARPGYRIIPESTVTEDIELGTIGEETPLLSEGVITAVEE
51 GAIGLPEVAIGVAGAIGTHAHEWWRDRYAFKSVLTGNYTDLKGNPLKPRN
101 AIPEKIKQLGKKIFQGDFNRAFPDNLKLETEKEKADLLRYYNHNRRLAGL
151 SEAYPQGKGYAYAKSQKVLEAERRGLTVPGYKYLGPGNSLNRGQPINQID
201 EDAKEHDEAYDKVKTSQEVSRADNTFVNKALDHVVNAINFKETPGNAFGA
251 AIGAIGIGTKQAIEKYSGVIYPSVSG

***Bombyx mori* Densovirus V1up**

1 MPRIHFPYHNYLGPGTDNFE KNPIDEDDAIARSHDLAYDKVTNHKEVFQA
51 DKQARDEFFTSFVHTGNVHSLIGGIGLGTKNLVEEHVLGKPLYG

***Casphalia extranea* Densovirus V1up**

1 MPRIHFPYHNYLGPGSDNFKKQPVDEDDAIARAHDLDYDKASSDKDIFKA
51 DKQARDEFFSSFVHSGNLHSLIGGLGLGAKNLVEEHVLGKSLYG

Adeno-associated Virus V1up

1 MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPGY
51 KYLGPFNGLDKGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEF
101 QERLKEDTSFGGNLGRAVFQAKKRVLEPLGLVEEPVKTAPGKKRPVEHSP
151 VEPDSSSGTGKAGQQPARKRLNFGQTGDADSVPDPQPLGQPPAAPSGLGT
201 NT

Figure 9

```
1     TTATATCCTT AGGATATAAA GAGCAAGCAC CCAGGCACTT CTTCTATTCT
51    GCCTCAGATC GTCGATATAA GCGGAGCTTT TCTGTGACTG TACGTAGTGT
101   ATTGATCTTC GTGTTCTGGA ACTCAGGTAT GTTGTTCGCT GCAGTAGAAG
151   CGCCTCCAGC ATGTACGTTC GAACCTCGTT CATTGCAACA GTTATCACTT
201   AGAACTTTTG TAGAAAATAC GGATTGGGAC GTAATAAATT TTCTCTATAA
251   AAATAATATGAA TTTTCTAGCA GGTTGTATTC ATCGATGTTT TATGAAAGAA
301   AATATCTGGA TAATCCGCAT GAATATCATG ATTGGTCAGT ACCAAATAAT
351   GTTCCCATTG AAACGTACAT AAAGATAATG AATTATCACA TGGAAGACGG
401   TATTCCATCA TTTGCTTTCC AACGCAATTT AGTGAATAGA TGTTTCATAC
451   AAGTGCATTT CTGTGCTCCT GGTACTTTTG AGGTTTAACA GCTCTAATTT
501   ATTTGTATAC CTTGTTATAA TACATTTGCG AGGTTTAACA GCTCTAATTT
551   CTATCGGTTT AAGAAGTTTG TTCTCGTTCG CGATCAAACA GTTATTGAAG
601   ATGATGAACT TATCGATTAT ATGCAATGTA GAAGTAATTG GTGTACTTGT
651   TGTAATACAA CATCATTATT TGCGATTAAA GATGGAACTC ATTTAGGCCC
701   GCAGTGGTTT AATAGTATAT ATCTCATGTG TAACGAAATT CCGTTATTAC
751   CTGTTGCAGA TGATAACTGA GTTAAACGAT GGAGCCAGTA CAAGCACAGG
801   ACAATCTGGA TGGAGTAGAG GAGACGTTAT GAAGACATTA
851   TGGGGCCTCT GCGAGAATCA ACCGTTGACC AATCCGCAAT GGTGGGCGGA
901   CATGCTGGAG AGTGTTACCA TTTCTCTGGA GGATCAGAAC ACGAACCTAG
951   CCTCAGATTA CGCACAATTA AAGAAAAGCT TCCTGAAGAT TATATCGCAC
1001  TGGCAGACAA TGTCAACGAA ATGGTTGGTC ACTTCATCTG CGGACTTAGA
1051  TCGAAGAGTT TCAGCTCTAC TCGGCGGTAC ATTTCAGACG TTGTGGTCAC
1101  GTCGTCTCCT GAACACGGTC GCAGTAGTGCT GGACTTCCTT AATAAGTGCG
1151  CAAGCACTTA CCCCGGAAGA CTACTACTCT GGGTCAACGA AGGAGACCAC
1201  ATCCACGTCG TCCACGACTG CCCGTACAGC GCTGGACAAT GTAGATGCAA
1251  GTTCTCAAAA ACGGAAGACT TCAAAAGGG ACTTCGCAAG CGTCTACGCA
1301  AGTCGAAGTT CATTACCGAA CTCGATGACA CCGACTGGGC AAATGTTTTC
1351  TTATACTTCA TTGTGTCAAA ATGGAAAAGC GAACGAGCAG TTTGGATTGA
1401  TGGAGCCTTA CGACGACTAC CGGATACAGG TGAAGGTGTA CGATGGACCG
1451  CGCTGTGCGA GCAATCCCGA CTACTACTGG AACAACAAGC TGCAAGAGAT
1501  GGATGTGACG GTATCGAAGA AGTCACAGAT TTGGAAGGAC ATCAACAACG
1551  CGTTTTGCGT GGCGTATACC AACCTCGAAG AAAACGGAGC AAGTTCCAGA
1601  CCTTCGCCGA AGAAGCAAAA GTACTATTCT CAAAATTCAT CTGTATTCCT
1651  GTGCGTGACG CTAGAATAAT AATTGATAGG AAGCATCCTA TGTATTTTGA
1701  ATTGCATGAT CCAAAAAATG AAAAGTTGTA TACTGCTGCT ATTTCTTTAT
1751  ATGTGCGTGA CATTAATGAA ATGTCTTTGA ATGATTCTCA CTTACAGACT
1801  TTAGAGAATA CATGTGTTTA TCAAGCTGGT AATATAGACC CTTTTGTTTT
1851  TTATCATAAT ATGGAAGATA GTGTTCACTT TCTTTATGAA CTTATCTTAT
1901  TTCAATGTAA TGGTAGTGAG GAAGGTGTGA AAGAATTACT AATGAATATT
1951  GTAGCTTGGT TTAATAGGTT AGGTTGGTAT ATGCCAACAA TTAATATGAA
2001  TGGGGAAAAA GAATATCAGC TTAACCCTAA AGTTAATACT GTATGTATAA
2051  TTGGTAATCA TAATTGTGGT AAGAATTATT TTTGGGACGC AGTTTGCTGT
2101  TTGGGTTTAA ATGTGGGTTT TCTTGGTAGA GTTAATAATA AAACTAACAA
2151  GTTTGCTTTG CAAGATTGTG TTCACAAACG AATTGTAATT GGTAATGAAA
2201  TATTCTTTGGA AGATGGAGCA AAGGAGGATA TGAAGAAATT ATGTGAGGGG
2251  TGTCCATTCA ATGTTAGCGT AAAACATCAA TCAGATGGAA TTGTTGCCAG
2301  GACACCTGTT TGCTTAATTA GTAACAATTG TATACCTATT GCATGTGATG
2351  CTCATTTTGT AGATGTAAGG ATGAAGGTAT TTTATTGGAG GCCATGTAGT
2401  TATTTGGCCA AATCAAGGAA GAAGCCGTAT CCACCGGCTG TTTTTGAATT
2451  AATGAAAATG TATGGAGTTG ATTGGAATAA AGTTTATTAA TTCCTTTTAT
2501  TACATCTTTC ATTTTTGTTG CAATCCCATA ATAGTACTCT TCCCAACTGC
2551  ATGAGTGTAC TCCAAAGTAG AACTGGATTA ATCTCGTTTA TCAGGAATAG
2601  TATTAGGACC GTTAAAAAGA GGTCTAATGG AAGGATATTG AAGTCGTACT

2651  TTGCAAGCAG CTTCAACAAT AAAGTAAGCT TGAGTGTCAG TAAAATTAGC
2701  ATTATTAAGA TTATCATCTA AATTAGCTGT TGTCAATGCA TGAACAGGTT
2751  TCACACCAAT ATGCAAACTA GGTTGAGCTT TTGGTGTATA AGATGGAAAA
2801  GCTCCTGCAT GAAGTATTTG ACTTTTTCA ATTTGAGTCG CAATAGTCCA
2851  TTCAGTATTT GATACTACAG TATCATTAAA TTTTTCTGAA TCCCCTCTTA
2901  AAATATTATT AGTCATAGTG AAATCAGCAG TTCGATACTG CGAGTTCCCT
2951  GTACCTGCTG GGCAACTAAG AGTTGAGTTA TTGGGTACTG ATTTATCGCC CCACCCACTG CGCTAGGTAC
3001  ACCAGTATAT ACAGCTGGTA TAGCTTTTTT AATTAAACCC AATTGTGGTT
3051  TATAATTATA TTCAACAATT TCTCCTGGAG GTCCATTACA TCTAATTTCA
3101  TTTACATGAG ATTGTAAACA TTCGTAACCC GGATTATCTG CCGTACCGTT
3151  CTGTTGAAGA ACAAGTGCGA AATAATTCTT TAATGGATAC GGAATACCGA
3201  ACTGATGACG TGGTACATGA TTGGGTACTG ATTTATCGCC GTAAAACTCT
3251  TTARCATAAT CCTCAAAAAG ATTTTCATCA CGTATTGCTC CAAGTTCATC
3301  TATGGATGAT ACAACCATTG GTTCATTAGC TTGAAATTCT TTAGGACGTA
3351  CGTCAATACC TTGTGTTTTT ATATTCAGGC CTGTAGCATG AAGAATAAAT
3401  TGATTTTGAT TTAGAGTAGC AAGACCAGTA TTAGAACTAT TTGTTTGAAA
3451  AGCAATACGA GGGGTAAATG CTGTTACCGG TACATTACAT TCTTCAATGC
3501  AACTTCCAGG TGGTAATTGA GCAAATTCAT TTGGATTCAT ATACAAGAAC
3551  GGTCGGTCGA CTGGTAAACG TCCCAAACTA GTAATAACGA AGTTGTCAAC
3601  ATATGTAACA TCGTTAACTG TCCTTGTTAT TGGAATTACA ACATTTGCAA
3651  GACCATAAGT TAAAAATCGA TGTACTTTGT TAAAGATAAT GAAATTGTTA
3701  TGCGGAGTAG TAATTGGTCT AGGAATTCGA ATAATCGCTT GTACTATCTA
3751  TTCACCTGCA ATATCTGCAC CTGCTCCCGG TAATGATGTA CCTGCCATTC
3801  TTGAACGTTT ACCACCACTA CTTTTAGGAG GACGACCACC ACCGCCGCGT
3851  TTGCCCTTTC CCTTGTCTTG TGTTTTGTCC GGGGTTATGG GAGGAGTTAA
3901  AGGAGCACTC ATGGACGAAG AATCACCTTC GTCAGGTGGA GTACTATCTA
3951  CAGGATTACT TACAGGTTCA GGATCTGGAT CTGTGGAACG TCCAGGTTGC
4001  GCTGCTAAAT CAGTAGTCGG ATGATGTTCC TGTGTAGTAG AAGGAAGTTG
4051  ATCTACGTCG AAGTCGTCAA TGTCTTCCGG ATTACCTTCA GGTAGCTCGT
4101  CTAATATATC ATCTGCAGTT GGAGTTGCAG GTGCGACTTC TTCTTCTTCT
4151  GCACGAGGAG GTTCTGGAAT AGGATGATCA ATTGGTAAAC CTCGACGTAC
4201  TCGAGCTAAT TGCCATTGTT CAACAGCGTA CCGCCGTTGA CCTTCATTTA
4251  AGTATTCCCA ATTATCACGT TCTTGTGGAT GAGGTTTCAG TGGGGGTGTC
4301  ATTACACTGA AAACAAAATA AAAAGTATCA GTACTCACGT TTTTAGGTT
4351  GTTGAGGATA TACAGGTCCA CCAATTACGT CTTCAATTAC ACGTTTAGCT
4401  TTAAGTCCGT ATTTTCCAAC AAATGCTTGC CACGTACCGG ATTTCTGGTA
4451  TTCCTCAGAA AAACGATGAA TTGCTTCGTC GTCCAATTTA TATACTTCAG
4501  TTTTGAAATC TTCTTCAGTA AAATACTGTG ATTTTGCACG ATGCAATAAG
4551  TCTTCGTACC CAAGATCGTG TTCCTTAGCA ATTTGATCAG TTTCTGATCG
4601  TGCAGGTTTA GGATCTATTG GATTACCAGG CCCCACGAAA TCAGTGCCAG
4651  GTAATACTGC ACCTTTGCTA CTCGTGTGAT CAATTAATTT TTTAATTCCG
4701  ATGCCGATTC CTGCGAGGGC TGCTCCTCCT GCTGCAGTTG CCGCGACAGT
4751  TCCAAGAGTT CCAGCTGTGC CTGCTGCTGC GCTACCTGCA GTAGCGCCTC
4801  CCAAAAGTCC TGTTGTTTCG GTAGCTGCTT CGCCGAGTTC AAGTGCTGTT
4851  TCCTCAACTC CAGTAGAGAA ACTTGTTCA GCTTCGGCTG CTCCTTCACC
4901  TGCGTTGATT TCGTCTTCCG CGATATCAAT GCCAGTTTCT TCTGGCCCTG
4951  CCGCCTCTTG GAAACTAGTT TCGCGAAGAG GTTCTGGTTC ATTTGTAGGT
5001  AGTCTGTCGT ACGATTCCAC CATCTCCCAA ATCTATTACG
5051  AAGTTTAGGT GTATTTGCTA AAGGTAAATT ATTAGTACCT AAACTGAAAT
5101  CACGCAACGT GAGATCTGTA AAGACGCCAG ACATTTATTA CTACGATGCT
5151  AAAACTTCCG CTTATATACC TTCTGACGTC AGCGAAATAG AAGAAGTGCC
5201  TGGGTGCTTG CTCTTTATAT CCTTCGGATA TAAA
```

Figure 10

***Acheta domesticus* Densovirus nucleic acid sequence containing PLA_2 motif**

```
  1  ATGTCTGGCG TCTTTACAGA TCTCACGTTG CGTGATTTCA GTTTAGGTAC
 51  TAATAATTTA CCTTTAGCAA ATACACCTAA ACTTCGTAAT AGATTTGGGA
101  GATGGTGGAA TCGTAGTCAC CCCTACGACA GACTACCTAC AAATGAACCA
151  GAACCTCTTC GCGAAACTAG TTTCCAAGAG GCGGCAGGGC CAGAAGAAAC
201  TCGCATTGAT ATCGCGGAAG ACGAAATCAA CGCAGGTGAA GGAGCAGCCG
251  AAGCTGAAAC AAGTTTCTCT ACTGGAGTTG AGGAAACAGC ACTTGAACTC
301  GGCGAAGCAG CTACCGAAAC AACAGGACTT TTGGGAGGCG CTACTGCAGG
351  TAGCGCAGCA GCAGGCACAG CTGGAACTCT TGGAACTGTC GCGGCAACTG
401  CAGCAGGAGG AGCAGCCCTC GCAGGAATCG GCATCGGAAT TAAAAAATTA
451  ATTGATCACA CGAGTAGCAA AGGTGCAGTA TTACCTGGCA CTGATTTCGT
501  GGGGCCTGGT AATCCAATAG ATCCTAAACC TGCACGATCA GAAACTGATC
551  AAATTGCTAA GGAACACGAT CTTGGGTACG AAGACTTATT GCATCGTGCA
601  AAATCACAGT ATTTTACTGA AGAAGATTTC AAAACTGAAG TATATAAATT
651  GGACGACGAA GCAATTCATC GTTTTTCTGA GGAATACCAG AAATCCGGTA
701  CGTGGCAAGC ATTTGTTGGA AAATACGGAC TTAAAGCTAA ACGTGTAATT
751  GAAGACGTAA TTGGTGGACC TGTATATCCT CAACAACCTA AAAAACGTGA
801  GTAC
```

***Mythimna loreyi* Densovirus nucleic acid sequence containing PLA_2 motif**

```
  1  ATGTCTTTCT ACACGAGCGG GTTAACACAT CGCGCGCGAC CTGGCTATCG
 51  TATAATACCA GAAAGTACTG CTACCGAAGA TATAGAATTA GGTACTATTG
101  GTGAAGAAAC TCCTTTATTA AGTGAAAGTG CTATTACTGC TGTAGAAGAA
151  GGTGCTATTG GAGTTCCAGA AGTTGCCGTT GGACTTGCTG GTGCTATAGG
201  AACACATGCT GACGTGTTAT ATAGAAATAG AAACGTATTT AAAAGTGTTT
251  TAACTGGAAA TTACACTGAT TTAAAAGGAA ATCCAATAAA ACAAAGAAAT
301  TCTATTCCTG AGAAGACAAA GCAATTAGGA AAAGGTATAT TTCAAGGTGA
351  TTTTAATCGT GCATTTCCTG AAGATTTAAA AACAGAAACT GAGCAAGAAA
401  AAAACGATTT ATTACGATAC TATAATCATA ATAGAAGACT AGCTGGTTTA
451  AGTGAAGCTT ATCCACAAGG AAAAGGATAC GCTTATGCTA AGAGTCAAAA
501  AGTATTAGAA GCTGAAAGAC GTGGATTAAC TGTTCCTGGA TATAAATATC
551  TTGGTCCTGG AAACTCACTT AATAGAGGTC AACCTACTAA TCAAATAGAC
601  GAAGACGCTA AAGAACACGA CGAAGCTTAC GATAAAGCAA AAACAAGTCA
651  AGAAGTAAGT GAAGCAGATA ATACATTTGT TAATAAGGCG TTAGATCACG
701  TGGTTAATGC TATCAATTTT AAAGAAACGC CTGGTAACGC TTTTGGTGCT
751  GCTATTGGAG CTATTGGAAT TGGAACTAAG CAAGCTATTG AAAAACACAG
801  TGGAGTAATC TACCCTTCTG TTTCAGGT
```

Figure 11

***Acheta domesticus* Densovirus amino acid sequence containing PLA_2 motif**

```
  1  MSGVFTDLTL RDFSLGTNNL PLANTPKLRN RFGRWWNRSH PYDRLPTNEP
 51  EPLRETSFQE AAGPEETRID IAEDEINAGE GAAEAETSFS TGVEETALEL
101  GEAATETTGL LGGATAGSAA AGTAGTLGTV AATAAGGAAL AGIGIGIKKL
151  IDHTSSKGAV LPGTDFVGPG NPIDPKPARS ETDQIAKEHD LGYEDLLHRA
201  KSQYFTEEDF KTEVYKLDDE AIHRFSEEYQ KSGTWQAFVG KYGLKAKRVI
251  EDVIGGPVYP QQPKKREY
```

***Mythimna loreyi* Densovirus amino acid sequence containing PLA_2 motif**

```
  1  MSFYTSGLTH RARPGYRIIP ESTATEDIEL GTIGEETPLL SESAITAVEE
 51  GAIGVPEVAV GLAGAIGTHA DVLYRNRNVF KSVLTGNYTD LKGNPIKQRN
101  SIPEKTKQLG KGIFQGDFNR AFPEDLKTET EQEKNDLLRY YNHNRRLAGL
151  SEAYPQGKGY AYAKSQKVLE AERRGLTVPG YKYLGPGNSL NRGQPTNQID
201  EDAKEHDEAY DKAKTSQEVS EADNTFVNKA LDHVVNAINF KETPGNAFGA
251  AIGAIGIGTK QAIEKHSGVI YPSVSG
```

Figure 12

P7

```
DNA: TGGTCTAGTTCGCATTATCCTCCGCATTGGACTGCG
 +1: W  S  S  S  H  Y  P  P  H  W  T  A
```

P6

```
DNA: GTGAATCAGTCTTATACTTCGACTTGGTTTTGGGCT
 +1: V  N  Q  S  Y  T  S  T  W  F  W  A
```

P8

```
DNA: TGGCCTCAGTTGTTTACTTTTCCGTGTTGTAATCCT
 +1: W  P  Q  L  F  T  F  P  C  C  N  P
```

P10

```
DNA: GGTCTGAAGATTTGGAGTTTGCCGCCGCATCATGGG
 +1: G  L  K  I  W  S  L  P  P  H  H  G
```

Figure 13

14/14

SEQUENCE LISTING

<110> Tjissen, Peter
Zadori, Zoltan

<120> VIRAL PHOSPHOLIPASE A2 ENZYMES,
ANTI-VIRAL AGENTS AND METHODS OF USE

<130> 255-123pct

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<141> 2001-06-28

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<212> PRT
<213> Galleria mellonella densovirus

<400> 1
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1 5 10 15
Arg Gly Gln Pro Ile Asn Gln Ile Asp Glu Asp Ala Lys Glu His Asp
20 25 30
Glu Ala Tyr Asp Lys Val Lys Thr Ser Gln Glu Val Ser Arg Ala Asp
35 40 45
Asn Thr Phe Val Asn Lys
50

<210> 2
<211> 54
<212> PRT
<213> Mythimna loreyi densovirus

<400> 2
Leu Thr Val Pro Gly Tyr Lys Tyr Leu Gly Pro Gly Asn Ser Leu Asn
1 5 10 15
Arg Gly Gln Pro Thr Asn Gln Ile Asp Glu Asp Ala Lys Glu His Asp
20 25 30
Glu Ala Tyr Asp Lys Ala Lys Thr Ser Gln Glu Val Ser Glu Ala Asp
35 40 45
Asn Thr Phe Val Asn Lys
50

<210> 3
<211> 54
<212> PRT
<213> Junonia coenia densovirus

<400> 3
Leu Thr Val Pro Gly Tyr Lys Tyr Leu Gly Pro Gly Asn Ser Leu Asn
1 5 10 15
Arg Gly Gln Pro Thr Asn Gln Ile Asp Glu Asp Ala Lys Glu His Asp
20 25 30
Glu Ala Tyr Asp Lys Ala Lys Thr Ser Gln Glu Val Ser Gln Ala Asp
35 40 45
Asn Thr Phe Val Asn Lys
50

<210> 4
<211> 54
<212> PRT
<213> Pseudoplusia includens densovirus

<400> 4
Leu Thr Val Pro Gly Tyr Lys Tyr Leu Gly Pro Gly Asn Ser Leu Asp
1 5 10 15
Arg Gly Glu Pro Val Asn Gln Ile Asp Ala Asp Ala Lys Glu His Asp
20 25 30
Glu Ala Tyr Asp Lys Ala Lys Thr Ser Gln Glu Val Ser Asp Ala Asp
35 40 45
Ser Lys Phe Val Ser Lys
50

<210> 5
<211> 54
<212> PRT
<213> Diatraea saccharalis densovirus

<400> 5
Leu Thr Val Pro Gly Tyr Lys Tyr Leu Gly Pro Gly Asn Ser Leu Asn
1 5 10 15
Arg Gly Pro Pro Thr Asn Glu Ile Asp Ala Asp Ala Lys Glu His Asp
20 25 30
Glu Ala Tyr Ser Gln Ser Lys Thr Ala Gln Glu Val Ser Lys Ala Asp
35 40 45
Asn Thr Phe Val Asn Lys
50

<210> 6
<211> 54
<212> PRT
<213> Culex pipiens densovirus

<400> 6
Leu Val Pro Ala Pro Tyr Lys Tyr Ala Gly Pro Gly Asn Ser Leu Asn
1 5 10 15
Arg Gly Pro Ala Tyr Asp Leu Val Asp Glu Ser Ala Arg Gln His Asp
20 25 30
Ile Ala Tyr Asp Lys Ala Lys Ser Pro Glu Asp Ile His Lys Ala Asp
35 40 45
Arg Gln Phe Leu Thr Glu
50

<210> 7
<211> 54
<212> PRT
<213> Periplaneta fuliginos densovirus

<400> 7
Leu Thr Tyr Pro Phe His His Tyr Leu Gly Pro Gly Asn Pro Leu Asp
1 5 10 15
Asn Asn Glu Pro Val Asp Arg Asp Asp Ala Ile Ala Glu Glu His Asp
20 25 30
Lys Ala Tyr Ala Asn Ala Lys Ser Ser Ile Asp Val Ile Asn Ala Asp
35 40 45
Lys Lys Ala Ile Asp His
50

<210> 8
<211> 57
<212> PRT
<213> Acheta domesticus densovirus

<400> 8
Ala Val Leu Pro Gly Thr Asp Phe Val Gly Pro Gly Asn Pro Ile Asp
1 5 10 15
Pro Lys Pro Ala Arg Ser Glu Thr Asp Gln Ile Ala Lys Glu His Asp
20 25 30
Leu Gly Tyr Glu Asp Leu Leu His Arg Ala Lys Ser Gln Tyr Phe Thr
35 40 45
Glu Glu Asp Phe Lys Thr Glu Val Tyr
50 55

<210> 9
<211> 59
<212> PRT
<213> Casphalia extranea densovirus

<400> 9
Ile His Phe Pro Tyr His Asn Tyr Leu Gly Pro Gly Ser Asp Asn Phe
1 5 10 15
Lys Lys Gln Pro Val Asp Glu Asp Asp Ala Ile Ala Arg Ala His Asp

```
            20                  25                  30
Leu Asp Tyr Asp Lys Ala Ser Ser Asp Lys Asp Ile Phe Lys Ala Asp
        35                  40                  45
Lys Gln Ala Arg Asp Glu Phe Phe Ser Ser Phe
    50                  55
```

<210> 10
<211> 59
<212> PRT
<213> Bombyx mori densovirus

<400> 10

```
Ile His Phe Pro Tyr His Asn Tyr Leu Gly Pro Gly Thr Asp Asn Phe
 1               5                  10                  15
Glu Lys Asn Pro Val Asp Glu Asp Asp Ala Ile Ala Arg Ser His Asp
            20                  25                  30
Leu Ala Tyr Asp Lys Val Thr Asn His Lys Glu Val Phe Gln Ala Asp
        35                  40                  45
Lys Gln Ala Arg Asp Glu Phe Phe Thr Ser Phe
    50                  55
```

<210> 11
<211> 59
<212> PRT
<213> Canine parvovirus

<400> 11

```
Leu Val Pro Pro Gly Tyr Lys Tyr Leu Gly Pro Gly Asn Ser Leu Asp
 1               5                  10                  15
Gln Gly Glu Pro Thr Asn Pro Ser Asp Ala Ala Ala Lys Glu His Asp
            20                  25                  30
Glu Ala Tyr Ala Ala Tyr Leu Arg Ser Gly Lys Asn Pro Tyr Leu Tyr
        35                  40                  45
Phe Ser Pro Ala Asp Gln Arg Phe Ile Asp Gln
    50                  55
```

<210> 12
<211> 59
<212> PRT
<213> Mink enteritis parvovirus

<400> 12

```
Leu Val Pro Pro Gly Tyr Lys Tyr Leu Gly Pro Gly Asn Ser Leu Asp
 1               5                  10                  15
Gln Gly Glu Pro Thr Asn Pro Ser Asp Ala Ala Ala Lys Glu His Asp
            20                  25                  30
Glu Ala Tyr Ala Ala Tyr Leu Arg Ser Gly Lys Asn Pro Tyr Leu Tyr
        35                  40                  45
Phe Ser Pro Ala Asp Gln Arg Phe Ile Asp Gln
```

50 55

<210> 13
<211> 59
<212> PRT
<213> Mouse parvovirus 1

<400> 13
Leu Val Pro Pro Gly Tyr Lys Tyr Leu Gly Pro Gly Asn Ser Leu Asp
1 5 10 15
Gln Gly Glu Pro Thr Asn Pro Ser Asp Ala Ala Ala Lys Glu His Asp
20 25 30
Glu Ala Tyr Ala Ala Tyr Leu Arg Ser Gly Lys Asn Pro Tyr Leu Tyr
35 40 45
Phe Ser Pro Ala Asp Gln Arg Phe Ile Asp Gln
50 55

<210> 14
<211> 59
<212> PRT
<213> Feline panleukopenia parvovirus

<400> 14
Leu Val Pro Pro Gly Tyr Lys Tyr Leu Gly Pro Gly Asn Ser Leu Asp
1 5 10 15
Gln Gly Glu Pro Thr Asn Pro Ser Asp Ala Ala Ala Lys Glu His Asp
20 25 30
Glu Ala Tyr Ala Ala Tyr Leu Arg Ser Gly Lys Asn Pro Tyr Leu Tyr
35 40 45
Phe Ser Pro Ala Asp Gln Arg Phe Ile Asp Gln
50 55

<210> 15
<211> 59
<212> PRT
<213> Minute virus of mice

<400> 15
Met Val Pro Pro Gly Tyr Lys Tyr Leu Gly Pro Gly Asn Ser Leu Asp
1 5 10 15
Gln Gly Glu Pro Thr Asn Pro Ser Asp Ala Ala Ala Lys Glu His Asp
20 25 30
Glu Ala Tyr Asp Gln Tyr Ile Lys Ser Gly Lys Asn Pro Tyr Leu Tyr
35 40 45
Phe Ser Ala Ala Asp Gln Arg Phe Ile Asp Gln
50 55

<210> 16

<211> 59
<212> PRT
<213> Kilham rat parvovirus

<400> 16
Cys Val Pro Pro Gly Tyr Lys Tyr Leu Gly Pro Gly Asn Ser Leu Asp
1 5 10 15
Gln Gly Glu Pro Thr Asn Pro Ser Asp Ala Ala Ala Lys Glu His Asp
20 25 30
Leu Ala Tyr Asp Glu Tyr Ile Lys Ser Gly Lys Asn Pro Tyr Leu Tyr
35 40 45
Phe Ser Pro Ala Asp Gln Arg Phe Ile Asp Gln
50 55

<210> 17
<211> 59
<212> PRT
<213> Porcine parvovirus

<400> 17
Leu Thr Leu Pro Gly Tyr Lys Tyr Leu Gly Pro Gly Asn Ser Leu Asp
1 5 10 15
Gln Gly Glu Pro Thr Asn Pro Ser Asp Ala Ala Ala Lys Glu His Asp
20 25 30
Glu Ala Tyr Asp Lys Tyr Ile Lys Ser Gly Lys Asn Pro Tyr Phe Tyr
35 40 45
Phe Ser Ala Ala Asp Glu Lys Phe Ile Lys Glu
50 55

<210> 18
<211> 59
<212> PRT
<213> Muscovy duck parvovirus

<400> 18
Phe Val Leu Pro Gly Tyr Lys Tyr Val Gly Pro Gly Asn Gly Leu Asp
1 5 10 15
Lys Gly Pro Pro Val Asn Lys Ala Asp Ser Val Ala Leu Glu His Asp
20 25 30
Lys Ala Tyr Asp Gln Gln Leu Lys Ala Gly Asp Asn Pro Tyr Ile Lys
35 40 45
Phe Lys His Ala Asp Gln Glu Phe Ile Asp Asn
50 55

<210> 19
<211> 59
<212> PRT
<213> Goose parvovirus

<400> 19
Phe Val Leu Pro Gly Tyr Lys Tyr Leu Gly Pro Gly Asn Gly Leu Asp
1 5 10 15
Lys Gly Pro Pro Val Asn Lys Ala Asp Ser Val Ala Leu Glu His Asp
20 25 30
Lys Ala Tyr Asp Gln Gln Leu Lys Ala Gly Asp Asn Pro Tyr Ile Lys
35 40 45
Phe Asn His Ala Asp Gln Asp Phe Ile Asp Ser
50 55

<210> 20
<211> 59
<212> PRT
<213> Bovine parvovirus

<400> 20
Leu Thr Leu Pro Gly Tyr Asn Tyr Leu Gly Pro Phe Asn Ser Leu Phe
1 5 10 15
Ala Gly Ala Pro Val Asn Lys Ala Asp Ala Ala Ala Arg Lys His Asp
20 25 30
Phe Gly Tyr Ser Asp Leu Leu Lys Glu Gly Lys Asn Pro Tyr Leu Tyr
35 40 45
Phe Asn Thr His Asp Gln Asn Leu Ile Asp Glu
50 55

<210> 21
<211> 59
<212> PRT
<213> Simian parvovirus

<400> 21
Leu Thr Leu Pro Phe Ser Asn Tyr Ile Gly Pro Gly Asn Gln Leu Gln
1 5 10 15
Ala Gly Asn Pro Gln Ser Val Val Asp Ala Ala Ala Arg Ile His Asp
20 25 30
Phe Arg Tyr Ser Glu Leu Ile Lys Leu Gly Ile Asn Pro Tyr Thr His
35 40 45
Trp Ser Val Ala Asp Asp Glu Leu Leu His Asn
50 55

<210> 22
<211> 59
<212> PRT
<213> Chipmunk parvovirus

<400> 22
Ile His Leu Pro Ala Asp Arg Tyr Leu Gly Pro Gly Asn Pro Leu Glu
1 5 10 15
Asn Gly Pro Pro Val Asp Pro Val Asp Ala Val Ala Arg Ile His Asp

```
            20                  25                  30
Phe Arg Tyr Ala Asp Leu Glu Lys Gln Gly Ile Asn Pro Tyr Thr Thr
        35                  40                  45
Tyr Thr Ile Ala Asp Glu Glu Leu Leu Lys Asn
    50                  55
```

<210> 23
<211> 59
<212> PRT
<213> Adeno-Associated Virus 2 parvovirus

<400> 23

```
Leu Val Leu Pro Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp
 1               5                  10                  15
Lys Gly Glu Pro Val Asn Glu Ala Asp Ala Ala Ala Leu Glu His Asp
            20                  25                  30
Lys Ala Tyr Asp Arg Gln Leu Asp Ser Gly Asp Asn Pro Tyr Leu Lys
        35                  40                  45
Tyr Asn His Ala Asp Ala Glu Phe Gln Glu Arg
    50                  55
```

<210> 24
<211> 59
<212> PRT
<213> Adeno-Associated Virus 3B parvovirus

<400> 24

```
Leu Val Leu Pro Gly Tyr Lys Tyr Leu Gly Pro Gly Asn Gly Leu Asp
 1               5                  10                  15
Lys Gly Glu Pro Val Asn Glu Ala Asp Ala Ala Ala Leu Glu His Asp
            20                  25                  30
Lys Ala Tyr Asp Gln Gln Leu Lys Ala Gly Asp Asn Pro Tyr Leu Lys
        35                  40                  45
Tyr Asn His Ala Asp Ala Glu Phe Gln Glu Arg
    50                  55
```

<210> 25
<211> 59
<212> PRT
<213> Adeno-Associated Virus 4 parvovirus

<400> 25

```
Leu Val Leu Pro Gly Tyr Lys Tyr Leu Gly Pro Gly Asn Gly Leu Asp
 1               5                  10                  15
Lys Gly Glu Pro Val Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp
            20                  25                  30
Lys Ala Tyr Asp Gln Gln Leu Lys Ala Gly Asp Asn Pro Tyr Leu Lys
        35                  40                  45
Tyr Asn His Ala Asp Ala Glu Phe Gln Gln Arg
```

50 55

<210> 26
<211> 59
<212> PRT
<213> Adeno-Associated Virus 5 parvovirus

<400> 26
Leu Val Leu Pro Gly Tyr Asn Tyr Leu Gly Pro Gly Asn Gly Leu Asp
1 5 10 15
Arg Gly Glu Pro Val Asn Arg Ala Asp Glu Val Ala Arg Glu His Asp
20 25 30
Ile Ser Tyr Asn Glu Gln Leu Glu Ala Gly Asp Asn Pro Tyr Leu Lys
35 40 45
Tyr Asn His Ala Asp Ala Glu Phe Gln Glu Lys
50 55

<210> 27
<211> 59
<212> PRT
<213> Adeno-Associated Virus 6 parvovirus

<400> 27
Leu Val Leu Pro Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp
1 5 10 15
Lys Gly Glu Pro Val Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp
20 25 30
Lys Ala Tyr Asp Gln Gln Leu Lys Ala Gly Asp Asn Pro Tyr Leu Arg
35 40 45
Tyr Asn His Ala Asp Ala Glu Phe Gln Glu Arg
50 55

<210> 28
<211> 59
<212> PRT
<213> LuIII parvovirus

<400> 28
Trp Val Pro Pro Gly Tyr Lys Tyr Leu Gly Pro Gly Asn Ser Leu Asn
1 5 10 15
Gln Gly Glu Pro Thr Asn Pro Ser Asp Ala Ala Ala Lys Glu His Asp
20 25 30
Glu Ala Tyr Asp Gln Tyr Ile Lys Ser Gly Lys Asn Pro Tyr Leu Tyr
35 40 45
Phe Ser Pro Ala Asp Gln Arg Phe Ile Asp Gln
50 55

<210> 29

<211> 59
<212> PRT
<213> H1 parvovirus

<400> 29
Trp Val Pro Pro Gly Tyr Lys Tyr Leu Gly Pro Gly Asn Ser Leu Asp
1 5 10 15
Gln Gly Glu Pro Thr Asn Pro Ser Asp Ala Ala Ala Lys Glu His Asp
20 25 30
Glu Ala Tyr Asp Gln Tyr Ile Lys Ser Gly Lys Asn Pro Tyr Leu Tyr
35 40 45
Phe Ser Pro Ala Asp Gln Arg Phe Ile Asp Gln
50 55

<210> 30
<211> 59
<212> PRT
<213> B19 human parvovirus

<400> 30
Val Gln Leu Pro Gly Thr Asn Tyr Val Gly Pro Gly Asn Glu Leu Gln
1 5 10 15
Ala Gly Pro Pro Gln Ser Ala Val Asp Ser Ala Ala Arg Ile His Asp
20 25 30
Phe Arg Tyr Ser Gln Leu Ala Lys Leu Gly Ile Asn Pro Tyr Thr His
35 40 45
Trp Thr Val Ala Asp Glu Glu Leu Leu Lys Asn
50 55

<210> 31
<211> 35
<212> PRT
<213> Naja naja snake venom phospholipase A2 IA

<400> 31
Asp Phe Ala Asp Tyr Gly Cys Tyr Cys Gly Arg Gly Gly Ser Gly Thr
1 5 10 15
Pro Val Asp Asp Leu Asp Arg Cys Cys Gln Val His Asp Asn Cys Tyr
20 25 30
Asn Glu Ala
35

<210> 32
<211> 12
<212> PRT
<213> Naja naja snake venom phospholipase A2 IA

<400> 32
Ala Val Cys Asp Cys Asp Arg Leu Ala Ala Ile Cys

1 5 10

<210> 33
<211> 35
<212> PRT
<213> Human pancreatic phospholipase A2 IB

<400> 33
Glu Tyr Asn Asn Tyr Gly Cys Tyr Cys Gly Leu Gly Gly Ser Gly Thr
1 5 10 15
Pro Val Asp Glu Leu Asp Lys Cys Cys Gln Thr His Asp Asn Cys Tyr
20 25 30
Asp Gln Ala
35

<210> 34
<211> 12
<212> PRT
<213> Human pancreatic phospholipase A2 IB

<400> 34
Phe Ile Cys Asn Cys Asp Arg Asn Ala Ala Ile Cys
1 5 10

<210> 35
<211> 35
<212> PRT
<213> Human synovial fluid phospholipase A2 IIA

<400> 35
Ser Tyr Gly Phe Tyr Gly Cys His Cys Gly Val Gly Gly Arg Gly Ser
1 5 10 15
Pro Lys Asp Ala Thr Asp Arg Cys Cys Val Thr His Asp Cys Cys Tyr
20 25 30
Lys Arg Leu
35

<210> 36
<211> 12
<212> PRT
<213> Human synovial fluid phospholipase A2 IIA

<400> 36
Gln Leu Cys Glu Cys Asp Lys Ala Ala Ala Thr Cys
1 5 10

<210> 37

<211> 35
<212> PRT
<213> Gaboon viper snake venom phospholipase A2 IIB

<400> 37
Asp Tyr Ile Tyr Tyr Gly Cys Tyr Cys Gly Trp Gly Gly Lys Gly Lys
1 5 10 15
Pro Ile Asp Ala Thr Asp Arg Cys Cys Phe Val His Asp Cys Cys Tyr
20 25 30
Gly Lys Met
35

<210> 38
<211> 12
<212> PRT
<213> Gaboon viper snake venom phospholipase A2 IIB

<400> 38
Glu Leu Cys Glu Cys Asp Arg Val Ala Ala Ile Cys
1 5 10

<210> 39
<211> 35
<212> PRT
<213> Rat phospholipase A2 IIC

<400> 39
Ser Tyr Tyr Gly Tyr Gly Cys Tyr Cys Gly Leu Gly Gly Arg Gly Ile
1 5 10 15
Pro Val Asp Ala Thr Asp Arg Cys Cys Trp Ala His Asp Cys Cys Tyr
20 25 30
His Lys Leu
35

<210> 40
<211> 12
<212> PRT
<213> Rat phospholipase A2 IIC

<400> 40
Lys Ala Cys Glu Cys Asp Lys Leu Ser Val Tyr Cys
1 5 10

<210> 41
<211> 41
<212> PRT
<213> Bee venom phospholipase A2 III

<400> 41
Ile Ile Tyr Pro Gly Thr Leu Trp Cys Gly His Gly Asn Lys Ser Ser
1 5 10 15
Gly Pro Asn Glu Leu Gly Arg Phe Lys His Thr Asp Ala Cys Cys Arg
20 25 30
Thr His Asp Met Cys Pro Asp Val Met
35 40

<210> 42
<211> 12
<212> PRT
<213> Bee venom phospholipase A2 III

<400> 42
Leu Ser Cys Asp Cys Asp Asp Lys Phe Tyr Asp Cys
1 5 10

<210> 43
<211> 35
<212> PRT
<213> Human phospholipase A2 V

<400> 43
Asn Tyr Gly Phe Tyr Gly Cys Tyr Cys Gly Trp Gly Gly Arg Gly Thr
1 5 10 15
Pro Lys Asp Gly Thr Asp Trp Cys Cys Trp Ala His Asp His Cys Tyr
20 25 30
Gly Arg Leu
35

<210> 44
<211> 12
<212> PRT
<213> Human phospholipase A2 V

<400> 44
Asn Leu Cys Ala Cys Asp Arg Lys Leu Val Tyr Cys
1 5 10

<210> 45
<211> 35
<212> PRT
<213> Human phospholipase A2 X

<400> 45
Ala Tyr Met Lys Tyr Gly Cys Phe Cys Gly Leu Gly Gly His Gly Gln
1 5 10 15
Pro Arg Asp Ala Ile Asp Trp Cys Cys His Gly His Asp Cys Cys Tyr

20 25 30
Thr Arg Ala
35

<210> 46
<211> 12
<212> PRT
<213> Human phospholipase A2 X

<400> 46
Leu Leu Cys Lys Cys Asp Gln Glu Ile Ala Asn Cys
1 5 10

<210> 47
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR Primer

<400> 47
agtgggtatc gctactaacc tacactc
27

<210> 48
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR Primer

<400> 48
gatctgtcat catccagtct tctatgc
27

<210> 49
<211> 172
<212> PRT
<213> Porcine parvovirus

<400> 49
Ala Pro Pro Ala Lys Arg Ala Arg Gly Leu Thr Leu Pro Gly Tyr Lys
1 5 10 15
Tyr Leu Gly Pro Gly Asn Ser Leu Asp Gln Gly Glu Pro Thr Asn Pro
20 25 30
Ser Asp Ala Ala Ala Lys Glu His Asp Glu Ala Tyr Asp Lys Tyr Ile
35 40 45
Lys Ser Gly Lys Asn Pro Tyr Phe Tyr Phe Ser Ala Ala Asp Glu Lys

```
    50                  55                  60
Phe Ile Lys Glu Thr Glu His Ala Lys Asp Tyr Gly Gly Lys Ile Gly
65                  70                  75                  80
His Tyr Phe Phe Arg Ala Lys Arg Ala Phe Ala Pro Lys Leu Ser Glu
                85                  90                  95
Thr Asp Ser Pro Thr Thr Ser Gln Gln Pro Glu Val Arg Arg Ser Pro
            100                 105                 110
Arg Lys His Pro Gly Ser Lys Pro Pro Gly Lys Arg Pro Ala Pro Arg
        115                 120                 125
His Ile Phe Ile Asn Leu Ala Lys Lys Lys Ala Lys Gly Thr Ser Asn
    130                 135                 140
Thr Asn Ser Asn Ser Met Ser Glu Asn Val Glu Gln His Asn Pro Ile
145                 150                 155                 160
Asn Ala Gly Thr Glu Leu Ser Ala Thr Gly Asn Glu
                165                 170
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<210> 50
<211> 516
<212> DNA
<213> Porcine parvovirus

<400> 50
gcgcctcctg caaaaagagc aagaggacta actctaccag gatacaaata ccttggtcca
60
ggaaactcac tagaccaagg agaaccaact aatccatcag acgccgcagc aaaagaacac
120
gacgaagcct acgacaaata cataaaatct ggaaaaaatc catacttcta cttctcagca
180
gctgatgaaa aattcataaa agaaactgaa cacgcaaaag actacggagg taaaattgga
240
cattacttct tcagagcaaa gcgtgccttt gctccaaaac tctcagaaac agactcacca
300
actacatctc aacaaccaga ggtaagaaga tcgccgagaa aacacccagg gtctaaacca
360
ccaggaaaaa gacctgctcc aagacatatt tttataaact tagctaaaaa aaaagctaaa
420
gggacatcta atacaaactc taactcaatg agtgaaaatg tggaacaaca caaccctatt
480
aatgcaggca ctgaattgtc tgcaacagga aatgaa
516

<210> 51
<211> 227
<212> PRT
<213> B19 human parvovirus

<400> 51
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Ser Ser Lys Glu Ser Gly Lys Trp Trp Glu Ser Asp Asp Lys Phe Ala
 1               5                  10                  15
Lys Ala Val Tyr Gln Gln Phe Val Glu Phe Tyr Lys Lys Val Thr Gly
            20                  25                  30
```

```
Thr Asp Leu Glu Leu Ile Gln Ile Leu Lys Asp His Tyr Asn Ile Ser
        35                  40                  45
Leu Asp Asn Pro Leu Glu Asn Pro Ser Ser Leu Phe Asp Leu Val Ala
    50                  55                  60
Arg Ile Lys Asn Asn Leu Lys Asn Ser Pro Asp Leu Tyr Ser His His
65                  70                  75                  80
Phe Gln Ser His Gly Gln Leu Ser Asp His Pro His Ala Leu Ser Ser
                85                  90                  95
Ser Ser Ser His Ala Glu Pro Arg Gly Glu Asn Ala Val Leu Ser Ser
            100                 105                 110
Glu Asp Leu His Lys Pro Gly Gln Val Ser Val Gln Leu Pro Gly Thr
        115                 120                 125
Asn Tyr Val Gly Pro Gly Asn Glu Leu Gln Ala Gly Pro Pro Gln Ser
    130                 135                 140
Ala Val Asp Ser Ala Ala Arg Ile His Asp Phe Arg Tyr Ser Gln Leu
145                 150                 155                 160
Ala Lys Leu Gly Ile Asn Pro Tyr Thr His Trp Thr Val Ala Asp Glu
                165                 170                 175
Glu Leu Leu Lys Asn Ile Lys Asn Glu Thr Gly Phe Gln Ala Gln Val
            180                 185                 190
Val Lys Asp Tyr Phe Thr Leu Lys Gly Ala Ala Ala Pro Val Ala His
        195                 200                 205
Phe Gln Gly Ser Leu Pro Glu Val Pro Ala Tyr Asn Ala Ser Glu Lys
    210                 215                 220
Tyr Pro Ser
225
```

<210> 52
<211> 678
<212> DNA
<213> B19 human parvovirus

<400> 52
agtaaagaaa gtggcaaatg gtgggaaagt gatgataaat ttgctaaagc tgtgtatcag
60
caatttgtgg aattttataa aaaggttact ggaacagact tagagcttat tcaaatatta
120
aaagatcatt ataatatttc tttagataat cccctagaaa acccatcctc tctgtttgac
180
ttagttgctc gtattaaaaa taaccttaaa aactctccag acttatatag tcatcatttt
240
caaagtcatg gacagttatc tgaccacccc catgccttat catccagtag cagtcatgca
300
gaacctagag gagaaaatgc agtattatct agtgaagact tacacaagcc tgggcaagtt
360
agcgtacaac tacccggtac taactatgtt gggcctggca atgagctaca agctgggccc
420
ccgcaaagtg ctgttgacag tgctgcaagg attcatgact ttaggtatag ccaactggct
480
aagttgggaa taaatccata tactcattgg actgtagcag atgaagagct tttaaaaaat
540

ataaaaaatg aaactgggtt tcaagcacaa gtagtaaaag actactttac tttaaaaggt
600
gcagctgccc ctgtggccca ttttcaagga agtttgccgg aagttcccgc ttacaacgcc
660
tcagaaaaat acccaagc
678

<210> 53
<211> 276
<212> PRT
<213> Galleria mellonella densovirus

<400> 53
Met Ser Phe Phe Lys Asn Gln Leu Ile His Arg Ala Arg Pro Gly Tyr
1 5 10 15
Arg Ile Ile Pro Glu Ser Thr Val Thr Glu Asp Ile Glu Leu Gly Thr
20 25 30
Ile Gly Glu Glu Thr Pro Leu Leu Ser Glu Gly Val Ile Thr Ala Val
35 40 45
Glu Glu Gly Ala Ile Gly Leu Pro Glu Val Ala Ile Gly Val Ala Gly
50 55 60
Ala Ile Gly Thr His Ala His Glu Trp Trp Arg Asp Arg Tyr Ala Phe
65 70 75 80
Lys Ser Val Leu Thr Gly Asn Tyr Thr Asp Leu Lys Gly Asn Pro Leu
85 90 95
Lys Pro Arg Asn Ala Ile Pro Glu Lys Ile Lys Gln Leu Gly Lys Lys
100 105 110
Ile Phe Gln Gly Asp Phe Asn Arg Ala Phe Pro Asp Asn Leu Lys Leu
115 120 125
Glu Thr Glu Lys Glu Lys Ala Asp Leu Leu Arg Tyr Tyr Asn His Asn
130 135 140
Arg Arg Leu Ala Gly Leu Ser Glu Ala Tyr Pro Gln Gly Lys Gly Tyr
145 150 155 160
Ala Tyr Ala Lys Ser Gln Lys Val Leu Glu Ala Glu Arg Arg Gly Leu
165 170 175
Thr Val Pro Gly Tyr Lys Tyr Leu Gly Pro Gly Asn Ser Leu Asn Arg
180 185 190
Gly Gln Pro Ile Asn Gln Ile Asp Glu Asp Ala Lys Glu His Asp Glu
195 200 205
Ala Tyr Asp Lys Val Lys Thr Ser Gln Glu Val Ser Arg Ala Asp Asn
210 215 220
Thr Phe Val Asn Lys Ala Leu Asp His Val Val Asn Ala Ile Asn Phe
225 230 235 240
Lys Glu Thr Pro Gly Asn Ala Phe Gly Ala Ala Ile Gly Ala Ile Gly
245 250 255
Ile Gly Thr Lys Gln Ala Ile Glu Lys Tyr Ser Gly Val Ile Tyr Pro
260 265 270
Ser Val Ser Gly
275

<210> 54

<211> 828
<212> DNA
<213> Galleria mellonella densovirus

<400> 54
atgtctttct tcaaaaatca gttgatacat cgcgcacgac ctggttatcg tataataccg
60
gaaagtactg ttactgaaga tattgaatta ggtactattg gtgaagaaac tccattgtta
120
agtgaaggtg ttattacagc tgtagaagaa ggtgctattg gattaccaga agttgctatt
180
ggtgtggctg gtgctattgg aacacatgca catgaatggt ggagagatag atacgctttt
240
aaatctgttt taactggtaa ttatactgat ttaaaaggaa atcctttaaa acctagaaac
300
gctattcctg aaaaaattaa acaactagga aagaaaatat ttcaaggaga ttttaatcgt
360
gcatttcctg ataatttaaa attggaaact gaaaaagaaa aagctgattt attaagatat
420
tataatcata atagaagatt agctggacta agtgaagctt atccacaagg gaaaggatat
480
gcttatgcta aaagtcaaaa agtattagaa gctgaacgac gtggattaac tgttcctgga
540
tataaatatc ttggtcctgg aaattcattg aatagaggtc aacctataaa tcaaatagac
600
gaagacgcta aagaacacga cgaagcttat gataaagtga aaacaagtca agaagtaagt
660
cgagcagata atacatttgt taataaagcg ttagatcacg tggttaatgc tattaatttt
720
aaagaaacac ctggtaacgc ttttggtgct gctattggag ctattggaat tggaactaag
780
caagctattg aaaaatatag tggagtaatc tacccttctg tttcaggt
828

<210> 55
<211> 94
<212> PRT
<213> Casphalia extranea densovirus

<400> 55
Met Pro Arg Ile His Phe Pro Tyr His Asn Tyr Leu Gly Pro Gly Ser
1 5 10 15
Asp Asn Phe Lys Lys Gln Pro Val Asp Glu Asp Asp Ala Ile Ala Arg
20 25 30
Ala His Asp Leu Asp Tyr Asp Lys Ala Ser Ser Asp Lys Asp Ile Phe
35 40 45
Lys Ala Asp Lys Gln Ala Arg Asp Glu Phe Phe Ser Ser Phe Val His
50 55 60
Ser Gly Asn Leu His Ser Leu Ile Gly Gly Leu Gly Leu Gly Ala Lys
65 70 75 80
Asn Leu Val Glu Glu His Val Leu Gly Lys Ser Leu Tyr Gly
85 90

<210> 56
<211> 282
<212> DNA
<213> Casphalia extranea densovirus

<400> 56
atgcctcgta ttcattttcc ttatcataat tatcttggtc cgggcagtga taactttaaa
60
aaacaaccag tagacgaaga cgacgcaata gccagagcac atgacctgga ttacgataaa
120
gcaagctctg ataaagacat tttcaaggct gataagcagg ctcgcgacga gtttttcagt
180
tcatttgtgc acagcggaaa cttgcatagt ttaattggtg gactaggact tggagctaaa
240
aatctagtag aagagcatgt actaggtaag tccttgtacg gt
282

<210> 57
<211> 94
<212> PRT
<213> Bombyx mori densovirus

<400> 57
Met Pro Arg Ile His Phe Pro Tyr His Asn Tyr Leu Gly Pro Gly Thr
 1   5   10   15
Asp Asn Phe Glu Lys Asn Pro Ile Asp Glu Asp Asp Ala Ile Ala Arg
            20   25   30
Ser His Asp Leu Ala Tyr Asp Lys Val Thr Asn His Lys Glu Val Phe
        35   40   45
Gln Ala Asp Lys Gln Ala Arg Asp Glu Phe Phe Thr Ser Phe Val His
    50   55   60
Thr Gly Asn Val His Ser Leu Ile Gly Gly Ile Gly Leu Gly Thr Lys
65   70   75   80
Asn Leu Val Glu Glu His Val Leu Gly Lys Pro Leu Tyr Gly
                85   90

<210> 58
<211> 282
<212> DNA
<213> Bombyx mori densovirus

<400> 58
atgcctcgta ttcattttcc ttatcataat tatcttggtc cgggtactga taactttgaa
60
aaaaatccaa tagacgaaga cgacgctatc gcgaggtcgc atgatttggc ttacgataaa
120
gtaaccaatc ataaggaagt ttttcaagct gataaacagg cccgtgacga gtttttttact
180
tcatttgtgc atactggaaa cgtgcatagt ttaattggcg gtattggact tggaactaaa
240

aatttggtag aagaacatgt actaggtaaa cccttgtacg ga
282

<210> 59
<211> 202
<212> PRT
<213> Adeno-associated virus

<400> 59
Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Thr Leu Ser
1 5 10 15
Glu Gly Ile Arg Gln Trp Trp Lys Leu Lys Pro Gly Pro Pro Pro Pro
20 25 30
Lys Pro Ala Glu Arg His Lys Asp Asp Ser Arg Gly Leu Val Leu Pro
35 40 45
Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
50 55 60
Val Asn Glu Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65 70 75 80
Arg Gln Leu Asp Ser Gly Asp Asn Pro Tyr Leu Lys Tyr Asn His Ala
85 90 95
Asp Ala Glu Phe Gln Glu Arg Leu Lys Glu Asp Thr Ser Phe Gly Gly
100 105 110
Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro
115 120 125
Leu Gly Leu Val Glu Glu Pro Val Lys Thr Ala Pro Gly Lys Lys Arg
130 135 140
Pro Val Glu His Ser Pro Val Glu Pro Asp Ser Ser Ser Gly Thr Gly
145 150 155 160
Lys Ala Gly Gln Gln Pro Ala Arg Lys Arg Leu Asn Phe Gly Gln Thr
165 170 175
Gly Asp Ala Asp Ser Val Pro Asp Pro Gln Pro Leu Gly Gln Pro Pro
180 185 190
Ala Ala Pro Ser Gly Leu Gly Thr Asn Thr
195 200

<210> 60
<211> 606
<212> DNA
<213> Adeno-associated virus

<400> 60
atggctgccg atggttatct tccagattgg ctcgaggaca ctctctctga aggaataaga
60
cagtggtgga agctcaaacc tggcccacca ccaccaaagc ccgcagagcg gcataaggac
120
gacagcaggg gtcttgtgct tcctgggtac aagtacctcg gacccttcaa cggactcgac
180
aagggagagc cggtcaacga ggcagacgcc gcggccctcg agcacgacaa agcctacgac
240
cggcagctcg acagcggaga caacccgtac ctcaagtaca accacgccga cgcggagttt

300
caggagcgcc ttaaagaaga tacgtctttt gggggcaacc tcggacgagc agtcttccag
360
gcgaaaaaga gggttcttga acctctgggc ctggttgagg aacctgttaa gacggctccg
420
ggaaaaaaga ggccggtaga gcactctcct gtggagccag actcctcctc gggaaccgga
480
aaggcgggcc agcagcctgc aagaaaaaga ttgaattttg gtcagactgg agacgcagac
540
tcagtacctg acccccagcc tctcggacag ccaccagcag ccccctctgg tctgggaact
600
aatacg
606

<210> 61
<211> 276
<212> PRT
<213> Mythimna loreyi densovirus

<400> 61

Met	Ser	Phe	Tyr	Thr	Ser	Gly	Leu	Thr	His	Arg	Ala	Arg	Pro	Gly	Tyr
1				5				10					15		
Arg	Ile	Ile	Pro	Glu	Ser	Thr	Ala	Thr	Glu	Asp	Ile	Glu	Leu	Gly	Thr
			20					25					30		
Ile	Gly	Glu	Glu	Thr	Pro	Leu	Leu	Ser	Glu	Ser	Ala	Ile	Thr	Ala	Val
		35					40					45			
Glu	Glu	Gly	Ala	Ile	Gly	Val	Pro	Glu	Val	Ala	Val	Gly	Leu	Ala	Gly
	50					55					60				
Ala	Ile	Gly	Thr	His	Ala	Asp	Val	Leu	Tyr	Arg	Asn	Arg	Asn	Val	Phe
65					70					75					80
Lys	Ser	Val	Leu	Thr	Gly	Asn	Tyr	Thr	Asp	Leu	Lys	Gly	Asn	Pro	Ile
				85					90					95	
Lys	Gln	Arg	Asn	Ser	Ile	Pro	Glu	Lys	Thr	Lys	Gln	Leu	Gly	Lys	Gly
			100					105					110		
Ile	Phe	Gln	Gly	Asp	Phe	Asn	Arg	Ala	Phe	Pro	Glu	Asp	Leu	Lys	Thr
		115					120					125			
Glu	Thr	Glu	Gln	Glu	Lys	Asn	Asp	Leu	Leu	Arg	Tyr	Tyr	Asn	His	Asn
	130					135					140				
Arg	Arg	Leu	Ala	Gly	Leu	Ser	Glu	Ala	Tyr	Pro	Gln	Gly	Lys	Gly	Tyr
145					150					155					160
Ala	Tyr	Ala	Lys	Ser	Gln	Lys	Val	Leu	Glu	Ala	Glu	Arg	Arg	Gly	Leu
				165					170					175	
Thr	Val	Pro	Gly	Tyr	Lys	Tyr	Leu	Gly	Pro	Gly	Asn	Ser	Leu	Asn	Arg
			180					185					190		
Gly	Gln	Pro	Thr	Asn	Gln	Ile	Asp	Glu	Asp	Ala	Lys	Glu	His	Asp	Glu
		195					200					205			
Ala	Tyr	Asp	Lys	Ala	Lys	Thr	Ser	Gln	Glu	Val	Ser	Glu	Ala	Asp	Asn
	210					215					220				
Thr	Phe	Val	Asn	Lys	Ala	Leu	Asp	His	Val	Val	Asn	Ala	Ile	Asn	Phe
225					230					235					240
Lys	Glu	Thr	Pro	Gly	Asn	Ala	Phe	Gly	Ala	Ala	Ile	Gly	Ala	Ile	Gly
				245					250					255	

Ile Gly Thr Lys Gln Ala Ile Glu Lys His Ser Gly Val Ile Tyr Pro
260 265 270
Ser Val Ser Gly
275

<210> 62
<211> 828
<212> DNA
<213> Mythimna loreyi densovirus

<400> 62
atgtctttct acacgagcgg gttaacacat cgcgcgcgac ctggctatcg tataatacca
60
gaaagtactg ctaccgaaga tatagaatta ggtactattg gtgaagaaac tcctttatta
120
agtgaaagtg ctattactgc tgtagaagaa ggtgctattg gagttccaga agttgccgtt
180
ggacttgctg gtgctatagg aacacatgct gacgtgttat atagaaatag aaacgtattt
240
aaaagtgttt taactggaaa ttacactgat ttaaaaggaa atccaataaa acaaagaaat
300
tctattcctg agaagacaaa gcaattagga aaaggtatat ttcaaggtga ttttaatcgt
360
gcatttcctg aagatttaaa aacagaaact gagcaagaaa aaaacgattt attacgatac
420
tataatcata atagaagact agctggttta agtgaagctt atccacaagg aaaaggatac
480
gcttatgcta agagtcaaaa agtattagaa gctgaaagac gtggattaac tgttcctgga
540
tataaatatc ttggtcctgg aaactcactt aatagaggtc aacctactaa tcaaatagac
600
gaagacgcta aagaacacga cgaagcttac gataaagcaa aaacaagtca agaagtaagt
660
gaagcagata atacatttgt taataaggcg ttagatcacg tggttaatgc tatcaatttt
720
aaagaaacgc ctggtaacgc ttttggtgct gctattggag ctattggaat tggaactaag
780
caagctattg aaaaacacag tggagtaatc tacccttctg tttcaggt
828

<210> 63
<211> 268
<212> PRT
<213> Acheta domesticus densovirus

<400> 63
Met Ser Gly Val Phe Thr Asp Leu Thr Leu Arg Asp Phe Ser Leu Gly
1 5 10 15
Thr Asn Asn Leu Pro Leu Ala Asn Thr Pro Lys Leu Arg Asn Arg Phe
20 25 30
Gly Arg Trp Trp Asn Arg Ser His Pro Tyr Asp Arg Leu Pro Thr Asn

```
        35                  40                  45
Glu Pro Glu Pro Leu Arg Glu Thr Ser Phe Gln Glu Ala Ala Gly Pro
    50                  55                  60
Glu Glu Thr Arg Ile Asp Ile Ala Glu Asp Glu Ile Asn Ala Gly Glu
65                  70                  75                  80
Gly Ala Ala Glu Ala Glu Thr Ser Phe Ser Thr Gly Val Glu Glu Thr
                85                  90                  95
Ala Leu Glu Leu Gly Glu Ala Ala Thr Glu Thr Thr Gly Leu Leu Gly
            100                 105                 110
Gly Ala Thr Ala Gly Ser Ala Ala Ala Gly Thr Ala Gly Thr Leu Gly
        115                 120                 125
Thr Val Ala Ala Thr Ala Ala Gly Gly Ala Ala Leu Ala Gly Ile Gly
    130                 135                 140
Ile Gly Ile Lys Lys Leu Ile Asp His Thr Ser Ser Lys Gly Ala Val
145                 150                 155                 160
Leu Pro Gly Thr Asp Phe Val Gly Pro Gly Asn Pro Ile Asp Pro Lys
                165                 170                 175
Pro Ala Arg Ser Glu Thr Asp Gln Ile Ala Lys Glu His Asp Leu Gly
            180                 185                 190
Tyr Glu Asp Leu Leu His Arg Ala Lys Ser Gln Tyr Phe Thr Glu Glu
        195                 200                 205
Asp Phe Lys Thr Glu Val Tyr Lys Leu Asp Asp Glu Ala Ile His Arg
    210                 215                 220
Phe Ser Glu Glu Tyr Gln Lys Ser Gly Thr Trp Gln Ala Phe Val Gly
225                 230                 235                 240
Lys Tyr Gly Leu Lys Ala Lys Arg Val Ile Glu Asp Val Ile Gly Gly
                245                 250                 255
Pro Val Tyr Pro Gln Gln Pro Lys Lys Arg Glu Tyr
            260                 265
```

<210> 64
<211> 804
<212> DNA
<213> Acheta domesticus densovirus

<400> 64
atgtctggcg tctttacaga tctcacgttg cgtgatttca gtttaggtac taataattta
60
cctttagcaa atacacctaa acttcgtaat agatttggga gatggtggaa tcgtagtcac
120
ccctacgaca gactacctac aaatgaacca gaacctcttc gcgaaactag tttccaagag
180
gcggcagggc cagaagaaac tcgcattgat atcgcggaag acgaaatcaa cgcaggtgaa
240
ggagcagccg aagctgaaac aagtttctct actggagttg aggaaacagc acttgaactc
300
ggcgaagcag ctaccgaaac aacaggactt ttgggaggcg ctactgcagg tagcgcagca
360
gcaggcacag ctggaactct tggaactgtc gcggcaactg cagcaggagg agcagccctc
420
gcaggaatcg gcatcggaat taaaaaatta attgatcaca cgagtagcaa aggtgcagta

480
ttacctggca ctgatttcgt ggggcctggt aatccaatag atcctaaacc tgcacgatca
540
gaaactgatc aaattgctaa ggaacacgat cttgggtacg aagacttatt gcatcgtgca
600
aaatcacagt attttactga agaagatttc aaaactgaag tatataaatt ggacgacgaa
660
gcaattcatc gtttttctga ggaataccag aaatccggta cgtggcaagc atttgttgga
720
aaatacggac ttaaagctaa acgtgtaatt gaagacgtaa ttggtggacc tgtatatcct
780
caacaaccta aaaaacgtga gtac
804

<210> 65
<211> 5234
<212> DNA
<213> Acheta domesticus densovirus

<400> 65
ttatatcctt aggatataaa gagcaagcac ccaggcactt cttctattct gcctcagatc
60
gtcgatataa gcggagcttt tctgtgactg tacgtagtgt attgatcttc gtgttctgga
120
actcaggtat gttgttcgct gcagtagaag cgcctccagc atgtacgttc gaacctcgtt
180
cattgcaaca gttatcactt agaacttttg tagaaaatac ggattgggac gtaataaatt
240
ttctctataa aaataatgaa ttttctagca ggttgtattc atcgatgttt tatgaaagaa
300
aatatctgga taatccgcat gaatatcatg attggtcagt accaaataat gttcccattg
360
aaacgtacat aaagataatg aattatcaca tggaagacgg tattccatca tttgctttcc
420
aacgcaattt agtgaataga tgtttcatac aagtgcattt ctgtgctcct ggtacttttg
480
gcttccctat tacagataat atttgtatac cttgttataa tacatttgcg aggtttaaca
540
gctctaattt ctatcggttt aagaagtttg ttctcgttcg cgatcaaaca gttattgaag
600
atgatgaact tatcgattat atgcaatgta gaagtaattg gtgtacttgt tgtaatacaa
660
catcattatt tgcgattaaa gatggaactc atttaggccc gcagtggttt aatagtatat
720
atctcatgtg taacgaaatt ccgttattac ctgttgcaga tgataactga gttaaacgat
780
ggagccagta caagcacagg acaatctgga tggagtagag gagacgttga aggactttat
840
gaagacatta tggggcctct gcgagaatca accgttgacc aatccgcaat ggtgggcgga
900
catgctggag agtgttacca tttctctgga ggatcagaac acgaacctag cctcagatta
960

cgcacaatta aagaaaagct tcctgaagat tatatcgcac tggcagacaa tgtcaacgaa
1020
atggttggtc acttcattcg cggacttaga tcgaagagtt tcagctctac tcggcggtac
1080
atttcagacg ttgtggtcac gtcgtctcct gaacacggtc gcagagtgct ggacttcctt
1140
aataagtgcg caagcactta ccccggaaga ctactactct gggtcaacga aggagaccac
1200
atccacgtcg tccacgactg cccgtacagc gctggacaat gtagatgcaa gttctcaaaa
1260
acggaagact tcaaaaaggg acttcgcaag cctctacgca agtcgaagtt cattaccgaa
1320
ctcgatgaca ccgactgggc aaatgttttc ttatacttca ttgtgtcaaa atggaaaagc
1380
gaacgagcag tttggattga tggagcctta cgacgactac cggatacagg tgaaggtgta
1440
cgatggaccg cgctgtgcga gcaatcccga ctactactgg aacaacaagc tgcaagagat
1500
ggatgtgacg gtatcgaaga agtcacagat ttggaaggac atcaacaacg cgttttgcgt
1560
ggcgtatacc aacctcgaag aaaacggagc aagttccaga ccttcgccga agaagcaaaa
1620
gtactattct caaaattgaa ctgtattcct gtgcgtgacg ctagaataat aattgatagg
1680
aagcatccta tgtattttga attgcatgat ccaaaaaatg aaaagttgta tactgctgct
1740
atttctttat atgtgcgtga cattaatgaa atgtctttga atgatttcta cttacagact
1800
ttagagaata catgtgttta tcaagctggt aatatagacc cttttgtttt ttatcataat
1860
atggaagata gtgttcactt tcttaatgaa cttatcttat ttcaatgtaa tggtagtgag
1920
gaaggtgtga aagaattact aatgaatatt gtagcttggt ttaataggtt aggttggtat
1980
atgccaacaa ttaatatgaa tggggaaaaa gaatatcagc ttaaccctaa agttaatact
2040
gtatgtataa ttggtaatca taattgtggt aagaattatt tttgggacgc agtttgctgt
2100
ttgggtttaa atgtgggttt tcttggtaga gttaataata aaactaacaa gtttgctttg
2160
caagattgtg ttcacaaacg aattgtaatt ggtaatgaaa tatctttgga agatggagca
2220
aaggaggata tgaagaaatt atgtgagggg tgtccattca atgttagcgt aaaacatcaa
2280
tcagatggaa ttgttgccag gacacctgtt tgcttaatta gtaacaattg tatacctatt
2340
gcatgtgatg ctcattttgt agatgtaagg atgaaggtat tttattggag gccatgtagt
2400
tatttggcca aatcaaggaa gaagccgtat ccaccggctg tttttgaatt aatgaaaatg
2460
tatggagttg attggaataa agtttattaa ttccttttat tacatctttc atttttgttg
2520

caatcccata atagtactct tcccaactgc atgagtgtac tccaaagtag aactggaata
2580
aatctggtta tcaggaatag tattaggacc gttaaaaaga ggtctaatgg aaggatattg
2640
aagtcgtact ttgcaagcag cttcaacaat aaagtaagct tgagtgtcag taaaattagc
2700
attattaaga ttatcatcta aattagctgt tgtcaatgca tgaacaggtt tcacaccaat
2760
atgcaaacta ggttgagctt ttggtgtata agatggaaaa gctcctgcat gaagtatttg
2820
actttttca atttgagtcg caatagtcca ttcagtattt gatactacag tatcattaaa
2880
tttttctgaa tcccctctta aaatattatt agtcatagtg aaatcagcag ttcgatactg
2940
cgagttccct gtacctgctg ggcaactaag agttgagtta ccacccactg cgctaggtac
3000
accagtatat acagctggta tagctttttt aattaaaccc aattgtggtt tataattata
3060
ttcaacaatt tctcctggag gtccattaca tctaatttca tttacatgag attgtaaaca
3120
ttcgtaaccc ggattatctg ccgtaccgtt ctgttgaaga acaagtgcga aataattctt
3180
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(21) International Application Number: PCT/CA01/00932

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/00924 A3

(54) Title: VIRAL PHOSPHOLIPASE A_2 ENZYMES, ANTI-VIRAL AGENTS AND METHODS OF USE

(57) Abstract: The present invention provides a novel class of phospholipase A_2 enzyme (PLA_2) from viruses and a method for identifying, isolating, purifying and characterizing enzymes of this class. The present invention includes viral PLA_2 proteins, nucleic acids, and antisense oligonucleotides, and the use of these molecules in screening methods for anti-viral agents, in decreasing the infectivity and/or replication of viruses and as research tools. The present invention further includes treatment or prevention of virus-associated diseases using viral PLA_2 inhibitors and the use of the viral PLA_2-encoding region to improve virus-based gene therapy vectors.

INTERNATIONAL SEARCH REPORT

Intr tional Application No
PCT/CA 01/00932

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N9/20 C12N15/55 C12N15/11 C12N7/01 C07K16/10
C12Q1/34

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, EMBASE, CHEM ABS Data, SEQUENCE SEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BERGERON J ET AL: "Genome organization of the Kresse strain of porcine parvovirus: Identification of the allotropic determinant and comparison with those of NADL-2 and field isolates." JOURNAL OF VIROLOGY, vol. 70, no. 4, 1996, pages 2508-2515, XP002184679 ISSN: 0022-538X * see Fig. 1 * the whole document --- -/--	1-33

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 December 2001	18/12/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hillenbrand, G

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ERDMAN DEAN D ET AL: "Genetic diversity of human parvovirus B19: Sequence analysis of the VP1/VP2 gene from multiple isolates." JOURNAL OF GENERAL VIROLOGY, vol. 77, no. 11, 1996, pages 2767-2774, XP001037602 ISSN: 0022-1317 * see Table 1, U38546 * the whole document	1-33
X	DATABASE EMBL/GENBANK/DDBJ 'Online! Accession numbers L32896 and Q90125, 29 November 1994 (1994-11-29) TIJSSEN, P.: "Organization and expression of the ambisense genome of densonucleosis virus of Galleria mellonella (GmDNV)" XP002184680 abstract	1-33
X	DATABASE EMBL/GENBANK/DDBJ 'Online! Accession numbers AB042597 and Q9JFY0, 23 May 2000 (2000-05-23) NONAKA, K. ET AL.: "Complete nucleotide sequence and genome organization of a newly isolated Bombyx densovirus, which is clearly different in ORF structure from BmDNV previously reported." XP002184681 abstract	1-33
X	WO 00 28004 A (RABINOWITZ JOSEPH E ;SAMULSKI RICHARD JUDE (US); UNIV NORTH CAROLI) 18 May 2000 (2000-05-18) the whole document	1-33

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 36-42, 44-62

Present claims 36-42 and 44-62 relate to anti-viral agents/peptides defined by reference to a desirable characteristic or property, namely the inhibition of viral phospholipase A2.

The claims cover all agents (and their uses) having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such products. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the claimed agents by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search of the claimed matter impossible. It appears that the claims embrace a multiplicity of known agents which may exhibit such an anti-viral activity. Thus, no meaningful search could be carried out.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int tional Application No
PCT/CA 01/00932

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0028004 A	18-05-2000	AU 1911100 A	29-05-2000
		EP 1135468 A1	26-09-2001
		WO 0028004 A1	18-05-2000